Preface

This historical review on River Blindness, onchocerciasis, is written in honour of (i) the 120-year anniversary of the Bernhard Nocht Institute for Tropical Medicine (BNITM), founded in 1900 in Hamburg in the sequel of a tremendous cholera outbreak that hit Hamburg in 1892, (ii) the 75-year anniversaries of Acta Tropica and (iii) the Swiss Tropical and Public Health Institute (Swiss TPH), both founded in 1944 in Basel by the renowned scientist of tropical medicine Rudolf Geigy (1902-1995). Geigy was the first director of the former Swiss Tropical Institute (STI) and simultaneously the first editor of Acta Tropica.

River Blindness caused by the tissue filaria Onchocerca volvulus was one focus of research in the BNITM over almost 60 years documented in about 300 publications. Consequently, in this historical review article we have cited major articles based on research conducted at the BNITM.

In addition to this historical review article on onchocerciasis two historical articles on 75 year of both, Acta Tropica and Swiss TPH, are jointly published in in one issue of Acta Tropica.

Review

Onchocerciasis (River Blindness) – more than a Century of Research and Control

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Abstract

This review summarises more than a century of research on onchocerciasis, also known as river blindness, and its control. River blindness is an infection caused by the tissue filaria Onchocerca volvulus affecting the skin, subcutaneous tissue and eyes and leading to blindness in a minority of infected persons. The parasite is transmitted by its intermediate hosts Simulium spp. which breed in rivers. Featured are history and milestones in onchocerciasis research and control, state-of-the-art data on the parasite, its endobacteria Wolbachia, on the vectors, previous and current prevalence of the infection, its diagnostics, the interaction between the parasite and its host, immune responses and the pathology of onchocerciasis. Detailed information is documented on the time course of control programmes in the afflicted countries in Africa and the Americas, a long road from previous programmes to current successes in control of the transmission of this infectious disease. By development, adjustment and optimization of the control measures, transmission by the vector has been interrupted in foci of countries in the Americas, in Uganda, in Sudan and elsewhere, followed by onchocerciasis eliminations. The current state and future perspectives for control, elimination and eradication within the next 20-30 years are described and discussed. This review contributes to a deeper comprehension of this disease by a tissue-dwelling filaria and it will be helpful in efforts to control and eliminate other filarial infections.
1. Introduction and history

Onchocerciasis – commonly known as river blindness – is an infectious disease caused by the parasitic filaria Onchocerca volvulus Leuckart 1893 (Parsons, 1908) belonging to the tissue-residing nematodes (class Chromadorea). The name Onchocerca derived from a combination of the Greek words ‘onchos’ meaning ‘hook’ and ‘kerkos’ meaning ‘tail’. The infection occurs primarily in sub-Saharan Africa, but it is also found in Yemen and until recently in foci of six countries within Central America (WHO, 1995; WHO 2020 fact sheets, 2019; WHO, 2020). The Global Burden of Disease Study 2010 (Hotez et al., 2014) quantified the burden of almost 300 diseases, including onchocerciasis, in terms of their relative impacts as disability-adjusted life years (DALYs) which was noted as 490,000 for onchocerciasis with 30 million infected people.

Primarily, the infection affects the skin and eyes and the pathology is caused by subcutaneous larvae (microfilariae). Microfilariae were first discovered in 1874 – almost 150 years ago – by John O’Neill, a British naval surgeon in the Gold Coast (Ghana), while examining skin-snips from so-called crow-crow patients suffering from intense acute dermatitis (O’Neill, 1875). Patrick Manson in 1890 first identified the adult microfilariae-releasing worms and in 1893 Rudolf Leuckart described their morphology from subcutaneous infestations as “Filaria volvuloxus”, now known as Onchocerca volvulus (Leuckart 1893; Fülleborn, 1908). The genus name Onchocerca had been given to filarial worms infecting cattle by Diesing in 1841. In 1917 Rodolfo Robles (Robles, 1917) published details of the association of dermatitis with subcutaneous nodules, microfilariae and anterior ocular lesions based on research in Guatemala and named the causative worms O. caecutiens (“blinding”) (Brumpt, 1919; Fülleborn, 1924). In 1927, while working in Sierra Leone, Blacklock discovered that blackflies, Simulium (Edwardsellum) damnosum Theobald transmitted O. volvulus causing onchocerciasis (Blacklock, 1927).

Chromosomes of Simulium were first described in 1937 (Painter et al., 1937) and those of the vectors in 1975 (Vajime and Dunbar, 1975), whereas for Onchocerca they were described later (Hirai et al., 1987; Post et al., 1989). The complete genome of O. volvulus was first published in 2016 by Choi et al. (2016) and Cotton et al. (2016). Rickettsiae-like endobacteria, Wolbachia, were first reported in Onchocerca in 1977 by Kozek and Figueroa-Marroquin (1977) and the genome of Wolbachia was described in 1999 by Bandi et al. (1999) and Slatko et al. (1999).

A control programme in 11 West African countries applying vector control began with the Onchocerciasis Control Programme (OCP) in 1974 and ended in 2002 (see section 4.2.2.). The OCP started with vector control by insecticide and was expanded to include treatment of Onchocerca-infected people with the microfilaricidal ivermectin in 1987. Treatment studies with doxycycline killing the Wolbachia endobacteria (Fig. 10) started in 2003 after initial in vitro experiments in 2000 (Hoerauf et al., 2000, 2001; Abegunde et al., 2016). Crump et al. (2012) described the onchocerciasis chronicle in detail and Table 1 summarises major milestones in the history of onchocerciasis research and control.
<table>
<thead>
<tr>
<th>Discovery</th>
<th>Characteristic</th>
<th>Discoverer/Originator</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin disease</td>
<td>&quot;Craw-craw&quot;</td>
<td>O'Neill</td>
<td>1875</td>
</tr>
<tr>
<td>Parasite</td>
<td><em>Filaria volvulus</em></td>
<td>Manson, Leuckart, Fülleborn, Railliet and Henry</td>
<td>1890, 1893, 1908, 1910</td>
</tr>
<tr>
<td></td>
<td>Nodule, onchocercoma</td>
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<td>Eye pathology, blindness</td>
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<td>Neurologic disease: epilepsy, nodding disease, dwarfism</td>
<td>Druet-Cabanac, Duke</td>
<td></td>
</tr>
<tr>
<td>Vector</td>
<td><em>Simulium</em></td>
<td>Blacklock, Vajime and Dunbar, Painter</td>
<td>1927, 1975, 1937</td>
</tr>
<tr>
<td>Phoretic host</td>
<td><em>S. damnosum</em> species complex: chromosomes</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td>Freshwater crab: <em>Potamonautes</em></td>
<td>van Someren</td>
<td>1950</td>
</tr>
<tr>
<td>Endobacteria</td>
<td><em>Simulium</em></td>
<td>Hertig and Wolbach, Kozek and Figueroa-Marroquin</td>
<td>1924, 1977</td>
</tr>
<tr>
<td><em>Wolbachia</em></td>
<td>Onchocerca</td>
<td></td>
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2. Parasite *Onchocerca*

2.1. *O. volvulus* life cycle

2.1.1 Development of the filaria in the human host

The infective third stage larvae (L3) of the parasite with a length of 600-700 µm invade the skin when an *O. volvulus*-infected blackfly bites the human host for blood (Fig. 1; Fig. 2). The L3 larvae moult in the skin of the human host to fourth stage larvae which migrate in subcutaneous tissue and grow to the adult female and male stages in 6-12 months. The adult females measure 30-60 cm, while the males are only 1.5-4.5 cm long (Fig. 2). The host reaction against the parasites leads to the formation of nodules known as onchocercomata. Interestingly, the males of the cattle parasite *O. ochengi*, and probably those of *O. volvulus* too, migrate between the nodules, thus from female to female.

In a single day an inseminated adult female can release 1000-3000 microfilariae responsible for the symptoms of the disease. The parasite has an enormous reproductive capacity resulting in millions of microfilariae released from fertilized females during their lives. Schulz-Key and Karam (1986) calculated that the number of microfilariae released from one female during its life of 10-15 years was >10 million.

The severity of the disease increases with the parasitic load and there is a direct relation between *O. volvulus* microfilarial load and host ocular morbidity (Little et al., 2004). The microfilariae leave the onchocercomata and move through the subcutaneous tissue to reach the cutis where they leave the host if taken up by a vector (Fig. 1).

2.1.2. Transmission by the vector *Simulium*

2.1.2.1. The genus *Simulium*

There are 2310 living species of blackflies in the family Simuliidae (order Diptera) and 18 known fossil taxa (Adler, 2019). Most species are of no economic importance but 1.5% of them are vectors of human pathogens causing onchocerciasis and mansonellosis (caused by *Mansonella ozzardi* in Brazil). Blackflies also transmit *Onchocerca* spp. to other vertebrates including cattle and, through their biting, they may also cause serious allergic reactions in cattle which can be fatal. In addition, they transmit protozoa to birds including *Leucocytozoon* spp. causing infections that are sometimes of economic importance for poultry. The major vectors of human onchocerciasis in Africa are members of the *S. damnosum* species complex of which at least 65 different forms have been described, with at least 15 of them acting as vectors (Adler et al., 2010).

In addition to their importance as vectors, blackflies can be serious biting pests of man and animals in both tropical and temperate climates, with examples including *S. erythrocephalum* in central Europe and *S. posticatum* in England. Particularly infamous was the Golubac fly *S. colombaschense* which in 1923 killed 22000 animals (sheep, goats, cattle) in the Danube valley in Yugoslavia, Hungary and Romania (Crosskey, 1990).
Members of the genus *Simulium* (Diptera: Simuliidae) have aquatic immature stages. The adults lay their eggs on trailing vegetation or rocks in streams or fast-flowing sections of rivers. Larvae hatch from the eggs and pass through 6 to 11 instars before becoming pupae from which the adults later emerge (Fig. 3). The males do not blood-feed but may obtain sugar-feeds from plants. Most female *Simulium* are haematophagous and it is this habit which links them to vectorial importance.

In Africa, the most important vectors are members of the *S. damnosum* species complex, but the disease is also transmitted in East Africa by members of the *S. neavei* complex (subgenus *Lewisellum*, 9 species) which has its immature stages phoretic on freshwater crabs. The most important vectors within the *S. neavei* complex are *S. neavei* in Uganda and *S. woodi* in Tanzania. In the Congo *S. albivirgulatum* is a vector in the central basin region (Fain et al., 1981). Recently *S. dentulosum* and *S. vorax* have been found biting man in the absence of *S. damnosum* s.l., *S. neavei* s.l. and *S. albivirgulatum* in the Ituri-Albert focus of the Democratic Republic of Congo. Furthermore, DNA tests confirmed the presence of *O. volvulus* in both *S. dentulosum* and *S. vorax* but few samples of the latter were available and none were infective. In contrast, 30% of 155 *S. dentulosum* were infected and 11% were infective, so a new vector species has been confirmed (R.J. Post et al. unpubl. and pers. comm.).

A variety of vectors is or was responsible for transmission in central and southern America. Members of the *S. ochraceum* and *S. metallicum* complexes were involved in Mexico, with the former most active in transmission in Guatemala (Garns and Ochoa, 1979a) before elimination of the disease in that country (Rodríguez-Pérez et al., 2015). In the remaining Amazonas focus that straddles the Venezuela-Brazil border the main vectors are members of the *S. oyapockense* and *S. guianense* species complexes and *S. incrustatum* (Shelley et al., 2010).

2.1.2.2. Development of the filaria in *Simulium*

The mouthparts of the female vectors of onchocerciasis do not penetrate the skin and absorb blood in the manner of mosquitoes but rather they use their mandibles to rasp at the skin until bleeding occurs and then the blood is lapped up from the resultant pool, hence they are known as pool-feeders (Fig. 3). Any *O. volvulus* microfilariae (Fig. 2.1) that are within the pool may be ingested by the blackflies. In vectors with pronounced buccopharangeal armatures such as members of the *S. ochraceum* complex, many microfilariae may be killed at this stage (Omar and Garms, 1975). In contrast, *S. metallicum* complex members lack such structures and if they ingest many microfilariae the flies may soon die shortly after their blood-meals (Omar and Garms, 1977), although this does not appear to happen with the *S. damnosum* complex which also lacks marked buccopharangeal armatures.

Once within the stomach with the blood meal the microfilariae must try to escape through the stomach wall into the haemocoel (Fig. 1) before being trapped within the peritrophic matrix that can form within 30 minutes. Bain et al. (1976) suggested that the reason that forest *S. damnosum* harbour higher numbers of developing *O. volvulus* larvae than do savannah forms was because of differences between the
two vector groups in the structure and speed of development of the peritrophic matrix, with forest flies’ membranes being less well sealed and slower to form.

Those microfilariae that reach the haemocoel move into the thoracic muscles, changing their shape to become “sausage stage” forms. These moult to the second stage (L2) and then moult again within the thoraces to become third stage larvae (L3) (Fig. 2.3) before emerging into the female’s body cavity to become elongated infective L3 stage larvae that will be capable of infecting a new host (Fig. 1, Fig. 2.3). L3s penetrate several fly organs, the ovaries, the brain, antennae, and the palps, thereby debilitating the fly resulting sometimes in only about 1% of the flies carrying L3s surviving, according to results of a laboratory study involving *S. yahense* (Trpis et al., 2006). Because the development process of the *Simulium* stages takes 6-9 days and the gonotrophic cycle of the females is 2-4 days, it is usually only at their third bite that the flies can transmit infections.

Much of what is known about the transmission of onchocerciasis by different *S. damnosum* complex members was derived from research conducted during the WHO Onchocerciasis Control Programme in West Africa (OCP). As mentioned in a later section (see section 4.2.2.), the OCP was affected by reinvasions of controlled zones by immigrant flies bred outside the treated areas. Thus, it became important to identify which members of the *S. damnosum* complex were responsible in order to be able to locate and treat the sources. Garms (1978) showed that savannah species could be separated from forest species by a combination of the colour of the basal wing tufts and the ratio between the lengths of the thoraces and antennae, with the latter being longer and less compressed in forest forms for a given fly size. Later morphological studies (Garms et al., 1982; Garms and Zillmann, 1984; Garms and Cheke, 1985; Meredith et al., 1983) allowed more of the species, or at least species groups, occurring in the OCP to be identified such that it was possible to compare their transmission abilities (Cheke and Garms, 2013). In general, forest forms carry more *O. volvulus* larvae per infected fly than the savannah forms.

2.2. Endobacterium *Wolbachia*

2.2.1. *Wolbachia* in the parasite *Onchocerca*

In 1977 Kozek and Figueroa-Marroquin (1977) found intracytoplasmic *Rickettsia*-like bacteria in *O. volvulus* and Sironi et al. (1995) provided molecular evidence for a close relative of the arthropod endosymbiont *Wolbachia* in a filarial worm. *Rickettsia*-like gram-negative endobacteria, alpha 2 proteobacteria, were originally detected in arthropods in 1924 by Hertig and Wolbach (1924), representing the eponym *Wolbachia* designated in 1936.

*Wolbachia* are found in the hypodermal cells of the lateral cords of adult filariae and in embryonic stages (Taylor and Hoerauf, 1999, Hoerauf et al., 2000) (Fig. 2.6). The absence of *Wolbachia* appears to be an ancestral condition (Bandi et al., 2001). The *Wolbachia* were shown to be mutualistic in *Onchocerca* (Comandatore et al., 2015), with the endobacteriae appearing to provide essential metabolites to the
filaria, which contribute to its reproduction and larval development, promoting fertility, embryogenesis and viability of the filaria (Comandatore et al., 2015).

Products of the endosymbionts have been implicated in the pathogenesis of ocular onchocerciasis (Saint André et al., 2002). Recent data support the hypothesis that suspected differences between severe and mild strains of *O. volvulus* (see section 2.3.) may be a function of their relative *Wolbachia* burden indicating that *Wolbachia* products may play a central role in the pathogenesis of ocular onchocerciasis (Pearlman, 2003).

The genome of onchocercal *Wolbachia* (*wOv*) has been analysed (Unnasch and Williams, 2000; Choi et al., 2016). The 956 kb genome contains 785 predicted protein-coding genes (http://exon.niaid.nih.gov/transcriptome/O.volvulus/v245/wOv_web/wOv_Web.xlsx) including the most abundant proteins: *Wolbachia* surface protein (*WSP, wOv00566*) and the chaperone DnaK (*wOv00687*) (Choi et al., 2016). The proteins were mapped to functional categories, with the top five functions being (i) translation, ribosomal structure, and biogenesis; (ii) post-translational modification, protein turnover, and chaperone; (iii) energy production and conversion; (iv) coenzyme metabolism and cell envelope biogenesis, and (v) outer membrane proteins (Bennuru et al., 2016).


2.2.2. *Wolbachia* in the vector *Simulium*

Yen (1975) first reported on intracellular *Wolbachia* in insects, long before the *Wolbachia* were identified as intracellular bacteria in filariae. *Wolbachia* are responsible for cytoplasmic incompatibility in *Culex pipiens* and numerous subsequent studies revealed that these endobacteria manipulate the reproduction of their arthropod hosts and can move horizontally across species’ boundaries. Meanwhile *Wolbachia* have been demonstrated in numerous mosquito vector species of medical and veterinary importance and have been used to control transmission of dengue fever by releasing *Aedes aegypti* vectors infected with the wMel strain of *Wolbachia* (Hoffmann et al., 2011).

The presence of *Wolbachia* in onchocerciasis vectors was first demonstrated by Crainey et al. (2010) who found the endosymbiont in larval samples of *Simulium* from Ghana. It is unclear if all individuals are infected, as the *Wolbachia* were found in less than a quarter of specimens, but they have also been detected in *S. squamosum* and *S. yahense* adults and in *S. oyapockense* s.l., with those in the latter differing markedly from the types found in the *S. damnosum* complex (J.L. Crainey, 2019, pers. comm). If there are consistent interspecific differences in the frequencies of occurrence and/or the varieties present in different vectors, it is possible, given *Wolbachia*’s known manipulation of reproductive capacities and vector status in other arthropods, that they could be of epidemiological importance, e.g. by accounting for differences in parasite burdens between forest and savannah vectors in West Africa. *Wolbachia* may also
be implicated in why *Simulium* are so difficult to colonise in the laboratory, e.g. by male-killing, and this
hurdle will need to be overcome if the potential of using *Wolbachia* in *Simulium* control can be realised.
Nevertheless, progress towards such a goal has been made with the discovery of a prophage element
within *S. squamosum* E that includes a SpvB-like protein at the extreme terminal end of its sequence
which is suspected of having insecticidal properties (Crainey et al., 2017).

2.3. Phylogeny and biology of *Onchocerca*

The phylogenetic tree of the genus *Onchocerca* Leuckart comprises 14 species divided into three
clades (Lefoulon et al., 2017). The third clade is composed of *O. volvulus* and the related species *O. ochengi*,
*O. gibsoni* and *O. gutturosa* which parasitize domesticated bovids. *O. volvulus* is genetically most closely
related to *O. ochengi* (https://parasite.wormbase.org/Onchocerca_ochengi_prjeb1465/Info/Index). The *O.
volvulus* / *O. ochengi* sister relationship supports the scenario that the human parasite resulted from a host
transfer by the bovine *O. ochengi*, or its ancestor (Bain, 2002; Morales-Hojas et al., 2006), possibly during
the course of cattle domestication and hence within the last 10,000 years, with *O. ochengi* switching into
humans to become *O. volvulus* (Lefoulon et al., 2017).

On the basis of the genetic similarity of *O. volvulus* and *O. ochengi*, the bovine infection by *O. ochengi*
has become famous as a natural model or ‘analogue’ of human onchocerciasis (Trees et al., 2000;
Makepeace and Tanya, 2016). A multitude of experimental joint studies by the University of Ngaoundéré
and the University of Tübingen, Germany, demonstrated similarities in the stage-specific proteome
(Armstrong et al., 2016), in excretory-secretory (E/S) peptides (Eberle et al., 2015), in immune
recognition of ES proteins (Manchang et al., 2015), in cross-protection (Wahl et al., 1998), in cross-
vaccination (Achukqui et al., 2007), and in the conserved nature of circulating miRNA (Quintana et al.,
2015). Further, similarities in the immune antigen recognition pattern was reported in *O. ochengi*-infected
cattle and in an *O. volvulus*-infected chimpanzee (Graham et al., 2000) confirming the use of the primate as
a surrogate host for *Onchocerca* infection. The comparability of both infections, nevertheless, appears to
be limited since in the evolutionarily primordial *O. ochengi* infections the parasite is highly adapted to its
bovine host eliciting minimal pathology, whereas in the evolutionarily younger parasitism by *O. volvulus*
severe damage often results (see section 3.3.). Further differences between the manifestation of onchocerciasis in forest and savannah regions, in particular
between blindness rates, gave rise to a two-strain hypothesis (Duke et al. 1966). Analyses of entomological
data (Cheke and Garms, 2013) and a reanalysis of pre-control blindness data (Cheke et al., 2020) have led
to this hypothesis being questioned. Although some molecular studies of different *O. volvulus* populations
revealed differences between savannah and forest strains, for instance by being distinguishable using the
0-150 repeat region sequence (Erttmann et al., 1987; Zimmermann et al., 1992), other studies did not
Recent studies involving nuclear DNA have not confirmed clear cut distinctions but did show that parasites from the two zones can and do interbreed (Choi et al., 2016). The complete genome of the mitochondria of *O. volvulus* ([https://parasite.wormbase.org/Onchocerca_volvulus_prjeb513/Info/Index](https://parasite.wormbase.org/Onchocerca_volvulus_prjeb513/Info/Index)) was published by Crainey et al. (2016) while the total genome of *O. volvulus* comprises a 97 Mb nuclear genome coding 12,143 protein-coding genes, and the onchocercal *Wolbachia* have a 956 kb genome containing 785 predicted protein-coding genes (Cotton et al., 2016; [http://parasite.wormbase.org/Onchocerca_volvulus_prjeb513/Info/Index](http://parasite.wormbase.org/Onchocerca_volvulus_prjeb513/Info/Index); [http://exon.niaid.nih.gov/transcriptome/O_volvulus/v245/wOv_web/wOv_Web.xlsx](http://exon.niaid.nih.gov/transcriptome/O_volvulus/v245/wOv_web/wOv_Web.xlsx)). Nine percent of the genes are *O. volvulus*-specific (Unnasch et al., 2000; Cotton et al., 2016; Choi et al., 2016). Recent reports on the transcriptome and proteome of *O. volvulus* and its *Wolbachia* endosymbiont (Bennuru et al., 2016) open up candidate molecules for diagnosis, new biomarkers, vaccine and drug targets. Further, the proteome of *O. volvulus* identified various mimics and antagonists of human cytokines and chemokines. Furthermore, the genome encodes numerous serine protease inhibitors such as serpins, as well as cysteine protease inhibitors like cystatin (Cotton et al., 2016). These proteins can interfere with antigen processing and presentation indicating parasite interference with host immune responses, thereby facilitating and promoting their survival in immunocompetent hosts. Interestingly, distinct encoded proteins are similar to human autoantigens, which may be implicated in the pathogenesis of eye diseases and nodding syndrome.

**Onchocerciasis is very probably an anthroponosis.** Humans are almost certainly the unique host of *O. volvulus* – although there are two reports indicating that primate can also host *O. volvulus*. Caballero and Barrera (1958) reported recovery of a nodule containing fertile *O. volvulus* adults from a golden spider monkey (*Ateles geoffroyi*) captured in Chiapas (Mexico), and a natural infection with *O. volvulus* has been found in a gorilla (*Gorilla gorilla*) by van den Berghe et al. (1964) in the Congo. Neumann et al. (1964) reported experimental onchocercal ocular lesions in chimpanzees (*Pan troglodytes*) and these primates were used as surrogate hosts in experimental infections for a long period to investigate humoral and cell-mediated immune responses, vaccination and drug effects (Greene, 1987; Taylor et al., 1988; Soboslay et al., 1991; Prince et al., 1992) until 2013 when the USA’s National Institutes of Health (NIH) banned invasive research on chimpanzees (Knight, 2008).

### 3. Disease

#### 3.1. Prevalence

Estimates of prevalence vary substantially. Thus, the WHO stated for 1983 that globally 85.5 million people were at risk and about 37 million people were infected with *O. volvulus* (Amazigo et al., 2008), with 0.34 million of the infected blinded by onchocerciasis (WHO, 1987). For 1995, the WHO noted 123 million at risk, 17.7 million infected people and 0.27 million blinded people (WHO fact sheets, 2019, 2020; Fig. 4).
Onchocerciasis is almost exclusively (>99%) prevalent in 31 countries of sub-Saharan Africa, but about 20,000 infected persons live in Yemen (Büttner et al., 1982; Connor et al., 1983; WHO 2019). In terms of populations living in areas where more than 50% live in areas where the predicted nodule prevalence is greater than 20%, the main countries are the Democratic Republic of Congo with 23.3 million people, Nigeria (14.3 million), Ethiopia (5.9 million) and Cameroon (5.2 million) (Zouré et al., 2014). In central Africa – the Central African Republic, Gabon, Democratic Republic of Congo, Angola, Sudan, Ethiopia, Uganda, Nigeria, Cameroon – onchocerciasis is co-endemic with loiasis. Loiasis, caused by the filaria Loa loa and transmitted by horseflies (Chrysops dimidiatus and C. silaceus) affect the eyes (African eye worm) (Vinkeles Melchers et al., 2020). Loiasis coinfection is a major concern for onchocerciasis elimination in Africa (see 4.1.).

Onchocerciasis also occurred in 13 isolated foci in six countries of Latin America, infecting 97 200 people with 500,000 people at risk (Sauerbrey, 2018; CDC 2013). Active transmission currently is limited to two foci among Yanomami indigenes in adjacent border areas of Venezuela and Brazil (CDC, 2013) (see section 4.3.3.). The fact that O. volvulus populations of African savannah and Central America are genetically indistinguishable, indicates that onchocerciasis was introduced into America by the entry of infected Africans from the savannah (Crump et al., 2012).

3.2. Diagnostics
Tests for diagnosing onchocerciasis are summarised in Table 2. Onchocerciasis is primarily diagnosed clinically by detecting onchodermatitis, by subcutaneous nodules (onchocercomata), often located at the hips, and by detection of microfilariae in skin snips (microfilaridermia) requiring a microscopic examination (Picq et al., 1971; Albiez et al., 1988a; Alhassan et al., 2016). Skin patch testing (Mazzotti reaction) with diethylcarbamazine (DEC) can indicate microfilaridermia (Mazzotti, 1951). Awadzi et al. (2015) reported a clinical evaluation of a transdermal delivery technology-based patch for the diagnosis of O. volvulus infection via skin exposure to diethylcarbamazine. Ocular status can also help diagnosis, if pathological findings such as punctate keratitis or even microfilariae in the anterior chamber are noted (O’Day and Mackenzie, 1985).

Laboratory analyses have been used since 1975 (Bartlett et al., 1975) to detect serum antibodies against Onchocerca surface proteins, preferentially IgG4, in infected persons by applying enzyme-linked immunosorbent assays (ELISA), by a fluorescent antibody staining technique or more recently by rapid-format antibody card test (Bartlett et al., 1975; Weil et al., 2000). As target antigens numerous O. volvulus-specific proteins have been investigated, in particular low molecular weight antigens such as Ov16 in addition to numerous additional proteins or hybrid proteins (Ov33, Ov10, Ov20 (Ov-FAR-1), Ov-RAL-2, Ov7, OvSOD1, Ov-ENO, Ov103, Ov9.3, OvMSA-1) (Lucius et al., 1988; Lobos et al., 1991; Mpagi et al., 2000; Andrews et al., 2008; McNulty et al., 2015; Unnasch et al., 2018). However, the sensitivity and species
specificity of antigen recognition assays are mostly of limited value because of cross-reactions with proteins from other filariae.

Ov16 was identified as highly diagnostic, mostly applied antigen (Lobos et al., 1991; Denery 2010; Lont et al., 2017; Bennuru et al., 2020). Subsequently, peptide epitopes (OvMP-23, OvNMP-48; OvOC9384, OvOC198, and OvOC5528; rOvOC10469 and rOvOC3261) (Gonzalez-Moa, 2018; Lagatie et al., 2019; Bennuru et al., 2018) were applied in ELISA analysis verifying the sensitivity and specificity by receiver operating characteristic (ROC) analysis. The lateral flow rapid assay with Ov16 and rOvOC3261 was developed as the best antigen-antibody test with 94% sensitivity and applied as the current diagnostic tool to verify interruption of transmission of O. volvulus (Vlaminck et al., 2015; Unnasch et al., 2018).

Some molecular biological analyses with high specificity have been developed such as real time polymerase chain reaction RT-PCR and colorimetric loop-mediated isothermal amplification (LAMP, Alhassan, 2016). The amplification assay targets a repeated O. volvulus sequence O-150, present in the O. volvulus genome with a unit length of roughly 150 bp.

Recently circulating biomarkers, e.g. peptides (OvOC3261, N-acetyl-tyramine- O-glucuronide, NATOG) (Globisch et al., 2013) or lipids (phospholipid, glycerophosphorlipid) as well as micro RNA have been developed as promising proof-of-contact diagnostic tests (Quintana et al., 2015; Lagatie et al., 2016; Gonzalez-Moa et al., 2018; Bennuru et al., 2020; Macfarlane et al., 2020).
### Table 2. Diagnostic tests for detection of *O. volvulus* infection

<table>
<thead>
<tr>
<th>Test</th>
<th>Target</th>
<th>Principle</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>Microfilaria</td>
<td>Microscopic detection of microfilariae in a skin snip biopsy</td>
<td>Picq 1971, Alhassan 2016</td>
</tr>
<tr>
<td>Mazzotti patch test</td>
<td>Microfilaria</td>
<td>Microfilaria-induced inflammatory reaction by provocation with DEC</td>
<td>Mazzotti 1958, Awadzi 2015</td>
</tr>
<tr>
<td></td>
<td>Peptide epitope</td>
<td>Recognition by IgG</td>
<td>Lagatie 2019, Gonzalez-Moa 2018</td>
</tr>
<tr>
<td></td>
<td>Ov16 and rOvOC3261</td>
<td>Antigen recognition by a Point-of-Care lateral flow rapid assay</td>
<td>Steel 2015, Bennuru 2020</td>
</tr>
<tr>
<td>qPCR¹</td>
<td>Genes: 0-150, Cox1, miRNA³</td>
<td>DNA amplification qPCR, LAMP</td>
<td>Zimmermann 1994, Alhassan 2016, Macfarlane 2020</td>
</tr>
<tr>
<td>LAMP²</td>
<td>Genes: OvOC3261, OvOC9384, OvOC198, OvOC5528</td>
<td>Lateral flow immunoassay, Liquid chromatography tandem mass spectrometry method (LC-MS/MS)</td>
<td>Denery 2010, Globisch 2013, Bennuru 2018, Bennuru 2020</td>
</tr>
<tr>
<td>Biomarker</td>
<td>NATOG⁴, Phospholipids OvOC3261, OvOC9384, OvOC9087, OvOC835, OvOC2224</td>
<td>Lateral flow immunoassay, Liquid chromatography tandem mass spectrometry method (LC-MS/MS)</td>
<td>Denery 2010, Globisch 2013, Bennuru 2018, Bennuru 2020</td>
</tr>
</tbody>
</table>

¹ quantitative polymerase chain reaction, ² Loop-mediated isothermal amplification, ³ microRNA, ⁴ NATOG, N-acetyltyramine-O-glucuronide

#### 3.3. Pathology

The primary manifestation of an *O. volvulus* infection is itching of the skin. The skin affliction results from the migration of a myriad of microfilariae from the subcutaneous nodules (onchocercomata), harbouring fertilised adult female and male filariae, into the adjacent skin. In the course of the infection an acute papular rash develops into a chronic papular dermatitis which may be associated with lichenification, development of papules, atrophy, and depigmentation. The skin manifestation may
comprise so-called "leopard, elephant or lizard skin". In addition, oedema and lymphadenopathy can occur and so-called “hanging groins” (Puente et al., 2018).

A distinct variation of skin pathology (Fig. 5) expressing severe chronic onchodermatitis with dark black hyperpigmentation and plaques, designated as sowda(h) (arabic word “aswad” for black) (Fig. 5.3), was originally observed in the Yemen by Gasparini (1962) and subsequently investigated by numerous scientists (Büttner et al., 1982; Connor et al., 1983; Ottesen, 1995; Richard-Lenoble et al., 2001, Al-Kubati et al., 2018). Sowda patients were also found in other endemic countries including Nigeria, Sudan, Ethiopia, Liberia, Guatemala and Ecuador (see section 4.3.1.). Patients with sowda manifestations exhibited generally low densities of microfilariae (Büttner and Racz, 1983; Büttner, 1984; Connor et al, 1983; Richard-Lenoble et al., 2001).

Adult female worms induce an inflammatory response in the infected host and infiltration of immune cells leads to the formation of a granuloma and then a subcutaneous nodule, an onchocercoma (Burchard et al., 1979) (Fig. 5.6). Also, excreted filarial proteolytic, angiogenic and collagen-inducing proteins promote the formation of the nodule or connective tissue-degrading activity (Haffner et al., 1998). The onchocercomata harbour 2-20 fixed and clustering females and 1-10 males migrating from nodule to nodule.

The most aggravating pathology in onchocerciasis is represented by severe visual impairment afflicting 500,000 people and blindness occurring in approximately 270,000 persons rendering river blindness the second most frequent cause of infectious blindness (Albiez et al., 1981; Hall and Pearlman, 1999). Most affected are patients with onchocercomata in the upper part of the body, including the head when microfilariae invade the eyes. The host’s reaction to the infiltrated microfilariae initiate corneal opacities or punctate keratitis that can develop into corneal scarring and a sclerosing keratitis.

Rarely, a disfiguring manifestation involving retarded growth (dwarfism, Nakalanga syndrome) occurs in onchocerciasis patients (Duke, 1998). Further pathogenic features are varying neurological diseases, nodding syndrome and epilepsy associated with autoimmunity (Colebunders et al., 2017; Johnson et al., 2017).

3.4. Parasite–Host interaction

The parasite *Onchocerca*, residing and developing in a human host, can survive because its resilient cuticle surface resists the host’s efforts to cope with the invading parasite. In addition, the parasite synthesizes and releases a myriad of intercepting excretory/secretory (E/S) molecules, via extracellular vesicles or directly. These molecules include antioxidants, protease-inhibitors, carbohydrate- and lipid-binding molecules and cytokine regulators, which mitigate and detoxify the offending host’s components (Hewitson et al., 2009; Njume et al., 2019). *O. volvulus* microfilariae also release matrix-degrading serine and metalloproteases which can degrade components of the dermal extracellular matrix and elastic fibres
of host tissue, as observed in chronic onchocerciasis (Haffner et al., 1998). Vital secreted defence
compounds of the filaria represent antioxidants like superoxide dismutase, peroxidoxin and thioredoxin
peroxidase. Also, proteinase inhibitors, onchocystatin and serpin, are released as protection against host
immune attack (Henkle-Dührsen and Kampkötter, 2001; Schö nemeyer et al., 2001; Hewitson et al., 2009).

In the course of the infection the host elicits a sequence of defence mechanisms reviewed by Ottesen
(1995), Brattig (2004a) and Maizels et al. (2018). In response to the filarial antigens the B-lymphocytes of
the host produce antibodies, predominantly immunoglobulin G4 (IgG4) and IgE antibody isotypes (Brattig
et al., 1994; Garaud et al., 1996; Adjebimey and Hoerauf, 2010). The blocking IgG4 type antibodies
enhance the parasite’s potential for host response evasion by inhibiting detrimental reactions since (i)
IgG4 represent non-complement-fixing immunoglobulins and (ii) IgG4 cannot induce antibody-dependent
cell-mediated cytotoxicity (ADCC) (Adjebimey and Hoerauf, 2010). Hence, high IgG4 concentrations are
found in immunosuppressed patients with high microfilarial loads (Adjebemey and Hoerauf, 2010;
Ottesen, 1995).

The defence mechanisms also include cellular responses of the adaptive and innate immune system:
lymphocyte and granulocyte populations are activated to secrete cytokines and toxic compounds which
affect the parasite (Ottesen 1995; Maizels et al. 2018). In Onchocerca-infected individuals the T helper
lymphocyte populations Th1, Th2, Th17 and regulatory T cells (Treg) are stimulated - predominantly
occurring as a Th2-response (Brattig et al., 1987; Plier et al., 1995; Timmann et al., 2003; Allen and
Sutherland, 2014). The helminth initiated lymphocyte subsets produce the cytokines IL-4, IL-13, IL-5, IL-
10, and TGF-beta which subsequently initiate multiple reactions from the innate immune system (Turaga
et al., 2000; Brattig et al., 1997; 2002; Soboslay et al., 1999; Dötze et al., 2000; Hoerauf and Brattig, 2002).

Characteristic of the innate immune response are eosinophilic and neutrophilic granulocytes, mast
cells and alternatively activated macrophages (Brattig, 2004a; Maizels et al., 2018). Predominant
eosinophilic granulocytes are activated by IL-5, released from Th2 cells, and their infiltration in the tissue
is regulated by IL-4 and IL-13. Such activated eosinophils adhere and degranulate at the surface of
microfilariae (Fig. 7.1-2) and infective larvae (Fig. 7.3-4) (Medina-De la Garza et al., 1990; Strue et al.,
1990; Brattig et al., 1991; Abraham et al., 2004). Eosinophilic effector cells produce reactive oxygen
species and secrete, via extracellular granules, multiple toxic molecules including oxygen radicals,
eosinophil peroxidase, major basic proteins, eosinophil cationic proteins , eosinophil-derived neurotoxin
and cytokines such as IL-10 and even IL-13 (Tischendorf et al., 1992; Pearlman 1997; Weller and Spencer,
2017). In addition to the eosinophils, mast cells are also operative in the host responses against helminths
(Ottesen, 1995; Korten et al. 1998). The Th1-associated response of neutrophilic granulocytes reflect the
presence of the endobacteria (see section 3.5.) (Brattig et al., 2001; Tamarozzi et al., 2016). In addition to
anti-parasitic reactions, the innate immune system is involved in wound repair mechanisms (Weller and
Spencer, 2017).
These multifarious reactions result in an inflammatory or immunosuppressed status which affect both competitors (Mackenzie et al., 1985; Ottesen, 1995; Brattig, 2004a). Hence, the pathogenesis of onchocerciasis is considered to be a consequence of long-standing reciprocal reactions of both parasite and host. The genetic constitution of the host represents one basic factor determining the variability of the host reactivities (Meyer et al., 1994; Timmann et al., 2008), and the presentation of a spectrum of disease manifestations (Ottesen, 1995, Büttner, 1984; Lucius et al., 1986; Hoerauf et al., 2003a; Brattig, 2004a).

Patients with a hyperreactive form of onchocerciasis, Sowda(h) (see section 3.3.) exhibit a particular host-parasite interaction (Bartlett et al., 1978; Connor et al., 1983; Ottesen, 1995; Brattig, 2004a). The high inflammatory potential visibly manifests in the skin showing hyper-pigmentation, lesions, pruritus and lichenification (Fig. 5). This activated state corresponds with strong cellular immune responses.

Characteristic are Th2 and Th17-Th2 lymphocytes and their secreted products such as IL-13, IL-4 and IL-17; they are associated with low Treg cells (CD4+CD25hiFoxp3+) reactivity (Brattig et al., 1987; Hoerauf et al., 2002; Katawa et al., 2015). Furthermore, high numbers of eosinophilic granulocytes, together with their released toxic cell products, and mast cells occur in hyperreactive onchocerciasis (Medina-De la Garza et al., 1990; Rubio-de Krömer et al., 1995; Tischendorf et al., 1992; Hoerauf et al., 2002). Although only a small number of microfilariae occur in the skin, the lack of Treg cells results in uncontrolled inflammatory responses (Brattig 2004a; Hoerauf and Brattig, 2002; Katawa et al., 2015). Serologically, strongly increased antibody including autoantibody responses are characteristic of sowda patients (Brattig et al., 1994; Gallin et al., 1995) (see section 3.3.). In consequence, these strong immune responses are associated with the reported low level of microfilarial density (Omar et al., 1979; Büttner and Racz, 1982, 1983; Siddiqui and Khawajah, 1991).

One major option for an effective host immune response is a prophylactic vaccine against the parasite, notably that spurred on and advanced by Sarah Lustigman (Lustigman et al., 2002, 2018; Hotez et al., 2015; George et al., 2019). Vaccines are aimed at preventing infection by infective larvae (anti-L3), and/or reducing microfilariae thereby complementing the control or elimination of onchocerciasis. Numerous proteins released by the filariae have been investigated for their vaccine potential; these comprise Ov-103, Ov-RAL-2, Ov-CHI-1, Ov_ALT-1, Ov-B20, Ov28CRP, Ov-GAPDH (Steisslinger et al., 2015; Lagatie et al., 2018; Lustigman et al., 2018). The alum-adjuvanted vaccine consisting of Ov-103, expressed at the surface of microfilariae, and Ov-RAL-2, found in the hypodermis of infective larvae, have the potential of reducing the infection by inhibition of moulting and survival of larvae. The development of cytphilic antibodies against the antigens and of interleukins effect antibody-dependent cellular cytotoxicity (George et al., 2019). Further, an immunomics approach with serum samples from putatively immune individuals has been applied (Bennuru et al., 2016). Recently a multi-epitope subunit vaccine coding for selected B-cell and T-cell epitopes, was constructed representing a novel approach for generating a specific immune response thereby avoiding responses against other unfavourable epitopes in the complete antigen (Shey et al., 2019). Another optional vaccine consists of nanoparticles or the use of non-protein molecules such
as carbohydrates, like the specific glycoform of glycosyl-phosphatidylinositol, that can act as vaccine candidates, as indicated for microbes (Jaurique and Seeberger, 2017).

3.5. Role of Wolbachia in onchocerciasis

The Wolbachia endobacteria are obligatory symbionts contributing to the viability of the parasite, its growth and development. The endobacteria are transovarially transmitted like mitochondria to the next filarial generation. Thus, antibiotics deployed to antagonize the endobacterial symbiotic role result in disruption of embryogenesis in female filariae.

Immunologically, the Wolbachia stimulate innate and adaptive immune responses. The bacteria-derived surface-associated and released molecules play immunological and pathological roles in onchocerciasis. In particular, endotoxin-like molecules induce Th1-type inflammatory reactions as known in all gram-negative lipopolysaccharide-exposing bacteria (Brattig et al., 2000; 2004b). In contrast to the helminth-characteristic type 2 (Th2) and Th3 immune responses, the Wolbachia provoke bacteria-typical predominant type 1 (Th1) reactions. Neutrophils - characteristically activated against bacteria - accumulate within an onchocercoma at the surface of a female comprising a multitude of Wolbachia in the lateral cord (see section 3.3., Fig. 7.5) (Brattig et al., 2001; Tamarozzi et al., 2016) - but neutrophils are absent in the onchocercoma on the surface of a female when the onchocerciasis patient has been treated with antibacterial doxycycline eliminating the Wolbachia (Fig. 9) (Brattig et al., 2001; 2004; Pearlman, 2003; Saint André et al., 2002). Subsequently, high peripheral levels of TNF-alpha, IL-1 beta, IL-6, IL-8 and antibacterial acute phase reactants arise on site and in the circulation. The Wolbachia surface protein (WSP) and heat-shock protein induce Th1-associated cytokines, TLR2/4 and IgG1 antibody responses (Pearlman, 2003; Brattig, 2004b; Kamalakannan et al., 2012; Tamarozzi et al., 2016).

The endotoxin-like and other products of the Wolbachia initiate a major proinflammatory stimulus in the eye disease leading to keratitis. Wolbachia, in addition, are associated with the severity of adverse reactions after chemotherapy of onchocerciasis with anti-filarial drugs. Wolbachia thus represent a target for therapy (Saint Andre et al., 2002; Pearlman, 2003).

4. Onchocerciasis control and elimination programmes

4.1. Elimination of onchocerciasis by chemotherapy of infected patients

Table 3 summarises past, present and potential future therapeutic agents for treatment of onchocerciasis. The first therapeutic agent against parasitic infections was suramin (Germanin; Bayer AG), a complex compound with four aromatic benzene rings and a functional urea group, which was introduced in 1949 (Wilson and Wormall, 1949). Suramin is a micro- and macrofilaricde, i.e. it not only kills microfilariae but also adult filariae. Suramin damages the intestinal epithelium of the filaria. Suramin, however, is inherently dangerous because of its high protein-binding affinity and alteration of enzyme
function; thus, it carries the risk of dermatitis, diarrhoea, optic neuropathy, nephrotoxicity, and even the occasional death. A three-year study in the Onchocerciasis Control Programme advised against suramin treatment (Rolland et al., 1980) and it is contraindicated in pregnancy.

Diethylcarbamazine (DEC, Hetrazan. PharmaCompass) a piperazine derivative, has been used as therapy against onchocerciasis since 1950 (Ruiz Reyes, 1951). It is also a micro- and macrofilaricide affecting the neuromuscular system of the parasites and promotes cellular cytotoxicity mediated by immune factors. In addition, DEC provokes various side effects such as itching and urticaria (reactions to disintegrating microfilariae) facial swelling, headache, nausea, vomiting, fever, joint pain and anorexia. DEC is used in a patch test (Mazzotti) for detection of skin microfilariae (see section 3.2).

Kuesel (2016) reviewed the path from discovery of new compounds (see below) to their qualification for large scale use and the support of regulatory authorities provides for development of drugs for neglected tropical diseases.

Ivermectin (Mectizan®, Merck) a macrocyclic lactone, derived from Streptomyces, is an endectocide. In 2015, the Nobel Prize in physiology or medicine was jointly awarded to W. Campbell (University of Wisconsin) and S. Omura (Kitasato University) at the Karolinska Institute (Sweden) for their discovery and exploration of ivermectin and its mode of action, resulting in a novel therapy against onchocerciasis and other nematode infections (Aziz et al., 1982; Campbell et al., 1983, 2016; Omura, 2016; van Voorhis et al., 2015) (Fig. 8). This molecule binds to the inhibitory neurotransmitter GABA on neurons and muscles resulting in an irreversible activation of a chloride influx, in a hyperpolarization of the membrane, and in paralysis and death of microfilariae. Ivermectin expresses micro- but not macrofilaricidal activity, although it causes long-term sterility of the adult female worms. Ivermectin was introduced for anti-filarial treatment in 1981. Albiez et al. (1988b) showed that ivermectin was a more effective microfilaricidal agent than DEC that caused more frequent and severe side effects. Initially, ivermectin was administered once a year (150 µg/kg), but lately it is increasingly administered bi-annually (Frempong et al., 2016). A 3-monthly treatment with ivermectin even may be most effective to prevent the appearance of onchocercomata (Campillo et al., 2020). The 3-monthly treatments not only target microfilariae, but probably in addition the moultng of third to fourth stage larvae and possibly can affect immature adults suggesting a prophylactic effect. Further, Navarro et al. (2020) reviewed data on the safety of high doses of ivermectin (>400 up to 800 µg/kg) but did not exclude ocular adverse events.

Ivermectin is donated free of charge by the Mectizan Donation Program and was distributed amongst communities by the African Programme for Onchocerciasis Control (APOC) and by various Non-Governmental Organisations (NGOs) such as Sight Savers, Lions International Sight First Programme, The Carter Foundation and the Helen Keller Foundation. Latterly, the Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN) has responsibility for oversight of ivermectin distribution in Africa. The programme reaches more than 300 million people in the affected areas of 35 countries annually, with more than 3.4 billion treatments donated since 1987. (The Mectizan® Donation
The drug has rare adverse effects such as red eyes and dry and burning skin. However, ivermectin is contraindicated in persons with loiasis due to the risk of ivermectin-associated severe inflammation since treatment with ivermectin may result in adverse reactions in patients with both onchocerciasis and loiasis (Gardon et al., 1997) (see section 3.1). In a ‘test-and-treat’ (TNT) strategy a rapid test (LoaScope) has been introduced for loiasis-endemic areas identifying individuals with levels of Loa loa microfilaremia associated with a risk of post-ivermectin severe adverse events. LoaScope-positive individuals were excluded from ivermectin treatment (Boussinesq et al., 2018). Adverse effects after ivermectin treatment have been observed at a rate of about 9% with cases showing hypotension or dyspnoea (De Sole et al., 1989). Bockarie et al. (2013) discussed the option of preventive chemotherapy as a strategy for elimination of onchocerciasis by treating populations at risk, to prevent transmission or morbidity.

Moxidectin, a milbemycin macrocyclic lactone, related to ivermectin, has been used since 1995 as an anthelminthic in veterinary medicine against various Onchocerca species (Monahan et al., 1995). Opoku et al. (2018) conducted a randomised, controlled, double-blind phase 3 trial in the Democratic Republic of Congo, Ghana and Liberia and stated that skin microfilarial loads were lower after moxidectin treatment than after ivermectin treatment. Moxidectin would therefore be expected to reduce parasite transmission between treatment rounds more than ivermectin could, thus accelerating progress towards elimination. Moxidectin has microfilaricidal and embryostatic effects after a single dose and expresses a macrofilaricidal effect upon repeated doses. Several studies indicate that moxidectin has a higher efficacy than ivermectin (Awadzi et al., 2014). Besides moxidectin, also flubendazole and emodepside had been investigated as candidate drugs (Kuesel, 2016).

Recently, metabolic chokepoint compounds have been identified which were either produced or consumed by a single enzyme reaction. The respective checkpoint enzymes that govern these reactions have been investigated (Taylor et al., 2013). Inhibition of such enzymes either leads to a toxic accumulation or lack of a compound necessary for subsequent reaction. Taylor reported anti-filarial effects on Onchocerca microfilariae by perhexiline, a piperidine derivative affecting carnitine o-palmitoyltransferase and the fatty acid oxidation pathway. Most recently, benzimidazole-benzoxaborole hybrids, amide- or ketone-linked, termed 8a (AN8799) or 21 (AN15470), have been reported as promising macrofilaricidal agents tested to date in animal models (Akama et al., 2020).

Since 1998 antibiotic therapy has demonstrated depletion of Wolbachia endobacteria in O. volvulus and other filariae (Hoerauf et al., 2000, 2001). Doxycycline was proposed for treatment of onchocerciasis in addition to ivermectin since adult females were sterilized when the antibiotic killed the Wolbachia (Fig. 9). However, a general implementation of doxycycline for filariasis therapy was hardly feasible because of the frequency and duration of the required treatment of 100 or 200 mg daily for 4-6 weeks. Also, adverse reactions have been reported and no pregnant women and children can take doxycycline (Hoerauf et al.,
Since 2014 an Anti-Wolbachia Consortium (A-WOL) at the Liverpool School of Tropical Medicine, has been active among others in the field of antiwolbachial drug discovery to treat filarial infections. There are numerous ongoing studies on novel alternate drugs against Wolbachia with excellent potential. The tylosin analog ABBV-4083 (TylAMac), a macrolide antibiotic, is an inhibitor of bacterial protein synthesis. ABBV-4083 resulted in a >99% elimination of Wolbachia as measured 16 weeks after treatment initiation, blocking the embryogenesis and leading to a complete clearance of circulating microfilariae. ABBV-4083 expressed relatively low activity against microfilariae of L. loa. A successfully completed phase I clinical trial assessing the safety and tolerability of ABBV-4083 has provided encouraging findings to support advancement of ABBV-4083 to phase II clinical trials (von Geldern et al., 2019; https://www.dndi.org/diseases-projects/portfolio/abbv-4083/).

There are other attractive non-macrolid antibiotic anti-Wolbachial compounds (AWZ=anti-Wolbachia) such as the heterocyclic thienopyrimidine/quinazoline scaffold AWZ1066 and its enantiomers AWZ1066-S and –R expressing drug metabolism/pharmacokinetic features (Hong et al., 2019). AWZ1066S is a highly specific anti-Wolbachia candidate selected through a lead optimization programme focused on balancing efficacy, safety and drug metabolism/pharmacokinetic (DMPK) features of a thienopyrimidine/quinazoline scaffold derived from phenotypic screening. AWZ1066S shows superior efficacy to existing anti-Wolbachia therapies in validated pre-clinical models of infection and has DMPK characteristics that are compatible with a short therapeutic regimen of 7 days or less. This candidate molecule is well-positioned for onward development and has the potential to make a significant impact on communities affected by filariasis. Furthermore, some intriguing future anti-Wolbachial candidate molecules include the heterocyclic quinazolines CRB417 and CRB490 with excellent efficacy and properties (Bakowski and McNamara, 2019). Very recently, in vivo efficacy of boron-pleuromutilin AN11251 against Wolbachia in the rodent filarial nematode Litomosoides sigmodontis model has been demonstrated to be superior to doxycycline (Ehrens et al., 2020). Thus, AN11251 treatment resulted in a Wolbachia FtsZ/actin reduction of 94% compared to <40% with doxycycline.
Table 3. Therapeutic agents for treatment of onchocerciasis

<table>
<thead>
<tr>
<th>Target</th>
<th>Therapeutic agents</th>
<th>Originator / Operator</th>
<th>Start of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Onchocerca</em></td>
<td>Suramin</td>
<td>Wilson and Wormall</td>
<td>1949</td>
</tr>
<tr>
<td></td>
<td>Diethylcarbamazine, DEC (Hetrazan)</td>
<td>Ruiz Reyes</td>
<td>1951</td>
</tr>
<tr>
<td></td>
<td>Ivermectin</td>
<td>Aziz</td>
<td>1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Campbell</td>
<td>1983, 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ömura</td>
<td>2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nobel prize, Karolinska Institute, Sweden</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>van Voorhis</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td>Moxidectin, Milbemycin</td>
<td>Monahan</td>
<td>1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Opoku</td>
<td>2018</td>
</tr>
<tr>
<td><em>Wolbachia</em></td>
<td>Doxycycline</td>
<td>Hoerauf</td>
<td>2002</td>
</tr>
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<td></td>
<td>Doxycycline versus Ivermectin</td>
<td>Abegunde</td>
<td>2016</td>
</tr>
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<td></td>
<td>Tolosin A analog: ABBV-4083, TylAMac</td>
<td>von Geldern</td>
<td>2019</td>
</tr>
<tr>
<td></td>
<td>AWZ1066S, CRB490/417</td>
<td>Taylor</td>
<td>2019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hong</td>
<td>2019</td>
</tr>
</tbody>
</table>

4.2. Vector control

4.2.1. Vector control 1932-1974

The first known attempt to control onchocerciasis by vector control was in Mexico in 1932. This and many other vector control efforts by both vegetation removal and chemical applications were reviewed by Davies (1994). There were aerial treatments with the organochlorine *Dichloro-diphenyl-trichloro-ethane* (DDT) of the River Congo at Kinshasa, now in the Democratic Republic of Congo, from 1948 to 1952 which led to the temporary disappearance of *S. damnosum* s.l., after which the vector populations have never recovered to their pre-control levels. DDT was in addition successfully used in Kenya and Uganda. In 1943, bush-clearing led to the disappearance by 1947 of vectors from the small (42 km²) Riana focus in Kenya and, in 1946, the vector *S. neavei* was eliminated from a focus in the Kodera district of Kenya by dripping DDT into rivers (McMahon et al., 1958), even though it was not known until 1950 that the species’ immature stages were phoretic on crabs. Also, in Kenya, DDT was successfully used to eradicate onchocerciasis vectors from foci in Kissy/Kericho and North Nyaza. A similar success was achieved in neighbouring Uganda, when DDT was used again to eliminate *S. damnosum* s.l. from the Victoria Falls by 1973.
Other control programmes were maintained in West Africa, for instance in Côte d’Ivoire from 1965 to 1971. DDT was phased out in favour of temephos, which was first used for Simulium control in the Sanaga river, Cameroon, in 1972, and temephos was the insecticide of choice used by OCP from 1975 (see below). This was after it was realised that localised control was only effective in isolated foci, when a plan for the massive Onchocerciasis Control Programme in the Volta Basin of West Africa (OCP) was initiated (https://www.who.int/blindness/partnerships/onchocerciasis_OCP/en/).

4.2.2. The World Health Organization Onchocerciasis Control Programme in the Volta Basin of West Africa (OCP)

At a meeting in Tunis during 1-8 July 1968 on the feasibility of onchocerciasis control it was agreed to plan a control campaign covering seven countries around the Volta Basin of West Africa (WHO/ONCHO/69.75 Joint US-AID/OCCGE/WHO Technical Meeting on the Feasibility of onchocerciasis control. Tunis, 1-8 July 1968, Report). This led to the production of a proposal to the Governments of Dahomey (now the Republic of Benin), Ghana, Ivory Coast (= Côte d’Ivoire), Mali, Niger, Togo and Upper Volta (now Burkina Faso) for the initiation of a control programme using aerial applications of insecticide to the vector’s breeding sites in rivers (WHO, 1973).

The OCP programme was established in 1974 and spraying started in 1975, with the aim of interrupting transmission for twenty years to allow for all existing adult worms to die (WHO/OCP/1973). This would intend to protect areas previously abandoned due to the severity of the disease and allow re-population and increased agricultural production. It was thought at the time that 5.9 million people would have been infected in the above seven countries in 1975. Later the programme was extended to include Guinea, Guinea-Bissau, the western part of Mali, Senegal and Sierra Leone where a further 6.8 million people would have been infected, but revised estimates suggest that these were underestimates and that 17.8 million were infected in the 11 countries of the extended OCP (O’Hanlon et al., 2016).

Detailed descriptions of the history and the structure of the OCP, the methods, and the results during the first five years of the programme were provided by Walsh et al. (1978, 1979) and updated by Philippon et al. (1990). To assess the results of the vector control measures from November 1974 to October 1978 almost 1.2 million S. damnosum females were caught in over 52,000 man-days of catching and 674,000 flies were dissected to determine Annual Biting Rates (ABRs) and Annual Transmission Potentials (ATPs) (Walsh et al., 1978).

Up to 6000 km of rivers were sprayed weekly from the air in the original 7 countries in the dry seasons and 18,000 km in the rainy seasons. Eventually, vector control was expanded into the southern and western extension areas bringing the total OCP area to 1 235 000 km² with a population of 30 million and increasing the lengths of rivers under control to 50 000 km (Samba, 1994).

The OCP did not succeed without overcoming a variety of operational problems. The first of these was the continuing presence of adult flies from the starts of rainy seasons at treated sites lacking larvae or
pupae and it was deduced, and later shown by experimental treatments of potential sources, that there was a reinvasion of the treated zone by flies bred outside it (Garms et al., 1979b). It was later established that the flies involved in studies in Côte d'Ivoire, in the central OCP area, were mostly savannah members of the *S. damnosum* complex (*S. damnosum* s.str. and *S. sirbanum*) and that they could migrate enormous distances of up to 500 km (Baker et al., 1990). Furthermore, they were parous and many carried infective larvae so they were of epidemiological importance (Garms et al., 1979b). A similar phenomenon also occurred in the east of the OCP, where *S. squamosum* was additionally involved (Cheke and Garms, 1983), and in the west where savannah flies were found to migrate both northeastwards and southeastwards out of and into Guinea, respectively (Baker et al., 1990).

Despite supplementing insecticidal control with mass drug administration of the microfilaricidal compound ivermectin, onchocerciasis control was not complete in some areas. Some rivers continued to have *S. damnosum* s.l. larvae present after extensive treatment cycles and infective adult flies were still being caught. After the main OCP ceased operations in 2002 such areas, designated as special intervention zones, continued to be treated with insecticides until 2001).

The great success of the OCP by 2002 was to have freed for agriculture 250,000 km² of fertile land from the threat of onchocerciasis, 40 million people had been protected from the disease and 600,000 cases of blindness prevented in seven countries (WHO, 2002). After the cessation of OCP, responsibility for continuing onchocerciasis control was devolved to the eleven member countries’ governments.

### 4.2.3. The African Programme for Onchocerciasis Control (APOP)

In 1995 WHO had instigated the African Programme for Onchocerciasis Control (APOP) aiming to promote control (and from 2009 elimination) by establishing self-sustaining community-directed treatment with ivermectin (CDTI), and, where appropriate, vector control with environmentally safe methods. The participating 19 countries were the remaining non-OCP endemic countries: Angola, Burundi, Cameroon, Central African Republic, Chad, Democratic Republic of Congo, Equatorial Guinea, Ethiopia, Gabon, Kenya, Liberia, Malawi, Mozambique, Nigeria, Rwanda, Sudan, Tanzania and Uganda. APOP's vector control activities were restricted to a few isolated foci, notably in Bioko (Equatorial Guinea) (Traoré et al., 2009), Tanzania and Uganda (Garms et al., 2009; https://www.who.int/apoc/vector/en/). APOP terminated in December 2015 and WHO’s action on onchocerciasis control was subsumed in May 2016 within the Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN) that deals not only with onchocerciasis but also with lymphatic filariasis, loiasis, schistosomiasis, soil-transmitted helminthiasis and trachoma (http://espen.afro.who.int/; Hopkins, 2016).

### 4.3. Current status of onchocerciasis in selected countries

According to WHO (2017) onchocerciasis control with mass drug administration (MDA) was still continuing in 2017 in the African Region in Angola, Benin, Burkina Faso, Burundi, Cameroon, Central
African Republic, Chad, Congo, Côte d’Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Ethiopia, Ghana, Guinea, Guinea Bissau, Liberia, Malawi, Mali, Mozambique, Nigeria, Senegal, Sierra Leone, South Sudan, Togo, Uganda and the United Republic of Tanzania. In the Eastern Mediterranean Region, MDA continued in Sudan and Yemen. The results of evaluations of CDTI were separated into those campaigns that were reported as being where criteria for stopping CDTI had been met or were close to elimination, on track to elimination or showing unsatisfactory progress.

4.3.1. Africa

4.3.1.1. Guinea

The first research on onchocerciasis was conducted by staff of the Bernhard Nocht Institute in the 1960s. Knüttgen (1964) and Knüttgen and Büttner (1968) examined 18,634 people in northeastern Guinea and reported that 45.8% carried microfilariae and 24% had nodules, with blindness rates varying from 1.07 to 4.56%. Subsequently, Knüttgen (1971) and De Sole et al. (1991) summarized the epidemiological situation. Garms and Post (1966) were the first to report the presence of *S. damnosum* s.l. and later Garms and Vajime (1975) found that, of the then known cytospecies, *S. yahense*, *S. sanctipauli*, *S. soubrense*, *S. damnosum* s.str. and *S. sirbanum* were present. This information was updated by Boakye et al. (1998). Guinea was part of the western extension of the OCP and was identified as a source of flies reinventing northern Côte d’Ivoire and Burkina Faso, involving distances of up to 500 km (Baker et al., 1990; see section 4.2.2.). According to WHO 2018a,b in 2017 almost 7 million people in 24 districts still required MDA with a reported coverage of 72.3%.

4.3.1.2. Liberia

Liberia was never a member of the OCP but onchocerciasis and its vectors were studied extensively by teams from the Bernhard Nocht Institute for Tropical Medicine, which established the Liberian Research Unit field station at Bong town, in 1968. The extent of onchocerciasis in the country was described by Frentzel-Beyme (1973, 1975a,b). Interestingly, the clinical state of onchocerciasis known as sowda is frequent in Liberia (Darge and Büttner, 1995) (see section 3.3.). Garms and Vajime (1975a) showed that *S. damnosum* s.str. and *S. sirbanum* occurred in the savannah areas, while in the forest *S. yahense* and a variety of members of the *S. sanctipauli* sub-complex are present, of which *S. yahense* is the most important vector (Garms, 1987; Trpis, 2006). The form of the *S. sanctipauli* complex occurring in the St. Paul river was originally described by Vajime and Dunbar (1975) as *S. sanctipauli* s.str. but after re-examinations of cytotaxonomic material Post (1986) pointed out that its chromosome patterns actually showed it to be a form of *S. soubrense*.

Furthermore, those populations are genetically distinct from the Farmington form present in the Farmington river (Kashan and Garms, 1987; Güzelhan and Garms, 1991). In addition *S. sanctipauli* s.str. does occur in Liberia too, but only in forest/savannah mosaic habitats in the Gestos, Mano and Makona...
rivers. Also, *S. soubrense* B (= *S. leonense*) was recorded in the Farmington river (Güzelhan and Garms, 1991). Anthropogenic factors have influenced vector dissemination as extensive deforestation associated with iron mining activities allowed savannah forms (*S. damnosum* s.str. and *S. sirbanum*) to invade previously forested areas as far south as Bong (Garms 1987; Garms et al., 1991).

Treatments with ivermectin began in 1999 but Liberia has also been the site of successful trials of a new macro- and microfilaricide, moxidectin (Opoku et al., 2018; see section 4.1).

4.3.1.3. Cameroon

At the beginning of the 1990s it was estimated that 1,300,000 people were infected in Cameroon, of whom 26,000 were blind (WHO, 1995). Major advances in onchocerciasis research were made from a base at Kumba in Cameroon by Duke and his colleagues who developed the concept of *Onchocerca–Simulium* complexes involving forest and savannah strains of the parasite (Duke et al., 1966; Duke 1967a; Lewis and Duke, 1966).

Non-*volvulus* species of *Onchocerca* are more commonly found in *S. damnosum* s.l. in Cameroon than is usual in many other African countries (Duke 1967b). This necessitates care in the analysis of the results of *Onchocerca* parasites counted in the vectors. The most important of these is *O. ochengi*, a cattle parasite, the impact of which on the transmission of *O. volvulus* was discussed by Eisenbarth et al. (2016).

Detailed studies in Cameroon of the vectorial abilities of *S. damnosum* s.str. and *S. sirbanum* have been conducted (Renz 1987, Renz and Wenk 1987) and on *S. squamosum* B in the Sanaga valley by Demanou et al. (2003), who also discussed data from Kumba on *S. squamosum* A and C.

Studies in Cameroon have highlighted the slow progress towards elimination based on ivermectin. Katabarwa et al. (2013) described how only 3 of 11 health districts were close to elimination after 15 years of treatment. Continuing transmission and prevalence of up to 52.7% were also reported for areas in the southwest by Wanji et al. (2015a) after 10 years of ivermectin distribution by CDTI. In another study it was reported that 15.5% of 2,364 people had never taken ivermectin (Wanji et al., 2015b). Similar results with onchocerciasis remaining at mesoendemic levels in the Centre and Littoral Regions and in the Vina du Nord River Valley after 15 and 25 years of CDTI, respectively, were documented by Kamga et al. (2016) and Eisenbarth et al. (2016).

It is unlikely that a long-term solution to the control of biting fly numbers or of onchocerciasis will be possible in the long-term without complementary vector control. Whilst, in theory, ivermectin distribution will interrupt transmission if distribution is maintained at 100% coverage for more than 25 years, in practice this is unlikely to be achieved because of (a) insufficient coverage for logistic and management reasons; (b) lack of acceptability of the drug in loiasis areas; and (c) the emergence of resistance to ivermectin, as "non-responders" (defined as individuals with microfilaria (mf) counts in skin >10 mf/snip after nine or more rounds of ivermectin treatment) have already been found in Ghana (Dadzie et al. 2003, Awadzi et al. 2004), with further evidence for it in Cameroon (Bourginat et al 2007).
According to WHO (2018a,b), in 2017 more than 11 million people in 112 of 113 districts required MDA and 71% were treated.

4.3.1.4. Uganda

In Uganda, where approximately 1.4 million people had been infected with onchocerciasis (Ndyomugyenyi 1998) and the disease existed in 17 foci, about 2.8 million people required MDA in 2017 (WHO 2018a,b), but not in 8 formerly endemic districts after onchocerciasis had been eliminated by MDA and vector control. So far Uganda, together with Kenya, Equatorial Guinea, Sudan and Ethiopia are the only African countries where onchocerciasis foci have been eliminated. In Uganda onchocerciasis is transmitted by two vectors, both of which were described from there: S. damnosum Theobald 1903 and S. neavei Roubaud 1915 (Adler, 2019). The larvae of S. neavei develop in a phoretic association on freshwater crabs of the genus Potamonautes and S. neavei is or was the vector in most of the smaller isolated foci. Uganda was one of the first countries where large scale vector control projects were carried out. From 1951 to 1973 there were 11 vector control projects, all with DDT (Davies 1994). Particularly famous were the projects on the important Victoria Nile focus where S. damnosum was breeding in a series of 70 km of rapids below the Owen dam. The application of 1973 was completely successful (McCrae, 1978), no flies have been found up to the present day (Davies, 1994), but it had never been formally verified that the transmission had been stopped. However, a recent study showed a total of 2953 serum samples taken from children younger than ten years and tested using the Ov16 ELISA test (see section 3.2.) were all negative (Katabarwa et al., 2020). Fly catches were carried out at the historical catching sites for at least a year. No S. damnosum were collected, indicating that the former vector never came back. However, 854 Simulium adersi Pomeroy (Subgenus Meiloniellum) were caught, which all turned out to be negative when tested by PCR. S. adersi is not known to be a vector of onchocerciasis, but can be infected experimentally (Wegesa, 1970).

Of the original 17 onchocerciasis foci in Uganda (Fig. 10) in only one, the Lhubiriha focus in Kasese District bordering D.R. Congo (Fig. 10.2, focus 14; red) is transmission by S. kilibanum (S. damnosum complex) still continuing. Interruption of the transmission by S. damnosum has now probably been achieved in the Mid North Focus (Fig. 10.2, focus 14; light green), mainly by vector control using temephos, but also experimentally by clearing of vegetation, window traps and CDTI. Vector control was also primarily responsible for the elimination of the Kashoya-Kitomi S. neavei focus (Lakwo et al., 2017). In most Ugandan foci where onchocerciasis has been eliminated by CDTI and vector control, Simulium neavei was the vector. Many of such successes followed on from research and control work begun in 1991 in cooperation between the German Technical Cooperation Agency (GTZ), the Bernhard Nocht Institute for Tropical Medicine, Basic Health Services Project, and the Vector Control Unit of the Ministry of Health in Kabarole District (now Kabarole and Kyenjojo districts) in Western Uganda.
When annual distribution of ivermectin began in 1991 no vector control was planned, but treatments were accompanied by studies on the transmission by the vector *S. neavei*. After 4 years, there was no clear effect on the transmission and 1000 parous flies still had 151 infective larvae in their heads. In view of these results and at the request of the local government it was decided to enhance the effect by vector control. Before starting this, it had been confirmed that temephos, which had been used for 25 years in the OCP (see section 4.2.2.), could be used safely, in particular, without harming the phoretic host crabs *P. Aloysia sabaudiae* (Garms et al., 2017). After only a few monthly applications from mid-1995 to the end of 1996, *S. neavei* had disappeared from the main Itwara focus and never came back. Sub-foci on the Siisa and Aswa rivers took a bit longer, but no positive crabs or biting flies were seen any more throughout the focus after February 2003 (Garms et al., 2009; Michael et al., 2020).

Of especial interest was the Imaramagambo focus in south-western Uganda, where vector control had been planned, but when it turned out that there was no transmission anymore, it was noticed that the vector *S. neavei* and its phoretic host, the freshwater crab, had both disappeared, possibly because of runoff into rivers of agricultural chemicals used intensively on the nearby tea plantations (Katabarwa et al., 2016). The flies also disappeared from areas in the Ruwenzori valley to the northwest of the Itwara focus (Garms et al., 1994) and habitat changes have also contributed to reductions in transmission elsewhere in Uganda, as Fischer et al. (1997) reported a reduced prevalence of onchocerciasis following deforestation. In addition, by 2017 MDA was leading to interruptions or suspected interruptions of transmission in five other foci where *S. neavei* was the vector (Katabarwa et al., 2018). The Madi Mid North focus, where *S. damnosum* s.l. is the vector, is likely to be the most intractable area but details of the cytoform present in that region have not been published. In western Uganda the following *S. damnosum* cytoforms have been recorded: *S. kilibanum*, “Sebwe”, “Nkusi” and *S. pandanophilum*, of which only *S. kilibanum* is anthropophilic and of vectorial importance (Krüger et al., 1999).

Control of *S. damnosum* s.l. by removing the trailing vegetation upon which immature stages develop was attempted in Mexico from 1932 to 1940 without success, in D.R. Congo in the early 1940s with only partial success, and in Malawi in the early 1990s by destruction of the aquatic plant *Hydrostachys* sp. (M. J. Roberts, unpubl., Burnham 1992, Davis, 1994). Also, Baker and Abdelnur (1986a,b) showed that in a rocky breeding site of the Bussere River in south-western Sudan a small team armed with axes, saws and sickles could do much to reduce larval and pupal supports in the breeding sites caused by vegetation trailing in fast water flow. A similar strategy has also recently been applied in Uganda (Jacob et al., 2018; Smith et al. 2019) but it is unlikely to provide a long-term solution as it requires regular “slash and clear” and, besides, the vectors will adapt and breed on other substrates such as rocks, which they often use when no trailing vegetation is present. In addition, large torrential rivers and rapids, particularly those in the middles of wide rivers cannot be completely cleared.

4.3.1.5. Ghana
As mentioned in the historical introduction, Ghana was the site where onchocerciasis was first recorded (O’Neill, 1875). Crisp (1956) described the geographical extent and severity of the disease in the north and provided plans for a vector control campaign, while Waddy (1969) elaborated these with proposals that culminated in the OCP. Ghana was one of the original seven OCP countries, with the west of the country targeted at the outset in 1975. Most of the rest of the country north of the Volta Lake was included in Phase II soon afterwards, with areas south of the lake included from 1988 onwards as part of the southeastern extension. Most of the southwestern forested areas were not included in the vector control campaign, but were subject to MDA with ivermectin, following the first successful trials that were conducted in 1987 at Asubende on the River Pru (Remme et al., 1989).

The initial euphoria about possible elimination of onchocerciasis from Africa using ivermectin distributions was dealt a blow when incipient resistance to the drug was detected in Ghanaian patients (Awadzi et al., 2004). Later, cohorts of patients were found in the Brong-Ahafo and Northern Regions to be being re-populated with microfilariae sooner than was to be expected and these “non-responders” (Osei-Atweneboana et al., 2007) were also possibly harbouring resistant worms (Osei-Atweneboana et al., 2011).

Despite the activities of the OCP and subsequent continuations of ivermectin distributions, some transmission continues (Kutin et al., 2004, Garms et al., 2015), including in areas where there had been vector control (Lamberton et al., 2015, F.B.D. Vereigh, pers.comm.to RAC July 2019).

The ivermectin distributions were originally annual but it is now the policy in Ghana to distribute bi-annually. This has succeeded in reducing transmission and infection rates in some areas but not everywhere that has been studied (Frempong et al., 2016, F.B.D. Vereigh, pers. comm.). Another consideration relevant to continuing transmission is the lack of complete compliance with the drug distribution programmes. Agyemang et al. (2018) investigated compliance in the Upper Denkyira East Municipal area and reported that it was lower than given in official reports, with results ranging from 7 to 51% with an overall compliance of only 21%, even less than the 24.4% reported earlier by Kutin et al. (2004).

Ghana has a high diversity of S. damnosum cytoforms, including some sites where up to five different varieties could be found breeding sympatrically. Details of the vectors present and how their geographical distributions have varied from the 1970s until 2011 were summarized by Post et al. (2013), with fluctuations attributable to deforestation (Wilson et al., 2002), extinction due to vector control (Cheke et al., 2008) and pollution resulting from illegal gold-mining activities in rivers such as the Pra and Offin (Garms et al., 2015), although the mining (locally known as “Galamsy”) has now been curtailed by Government actions. According to WHO (2018a,b) in Ghana in 2017 of about 8 million infected people 4.4 million (54.6%) in 84 of 85 endemic districts received MDA.
Onchocerciasis is highly endemic in Ethiopia, with more than 20 million people infected or at risk (Anon, 2015). The disease is mostly found in southwestern, western and northwestern parts of the country, being particularly associated with coffee growing areas in the southwest and with cotton and oil seed farming areas in the northwest. The main vectors are members of the S. damnosum complex (Hadis et al. 2005), but S. ethiopiense, a member of the S. neavei group phoretic on crabs (Potamonautes antheus), is suspected of being a secondary vector in the southwestern midlands and the highlands where it is often sympatric with S. damnosum s.l. (White 1977).

Onchocerciasis control with ivermectin began in 2001 and by 2015 there were 18 CDTI project zones in the country (Anon 2015). At present the country has some areas in various stages of post treatment surveillance (PTS), for instance in the Metema area (see below; 4.3.1.7.). Onchocerciasis has disappeared from the Tigray region in the absence of any control measures, probably in the wake of human migrations and the establishment of commercial farming (Katabarwa et al., 2014b). In 2017, 17.5 million people required MDA, with 194 of 199 endemic districts receiving it (WHO 2018a,b).

4.3.1.7. Sudan and South Sudan

4.3.1.7.1. Sudan

There are three main areas in Sudan where onchocerciasis occurs or occurred. Principal amongst these was the Abu Hamed focus in River Nile State where the first case of the elimination of an onchocerciasis focus in Africa was achieved following ivermectin distribution (Zarroug et al., 2016), although it was probably assisted by the flooding of all of the western breeding sites of the vector in the River Nile by the construction of the Merowe dam (Zarroug et al., 2014). The vector there is a unique form, the hamedense form of S. damnosum (Higazi et al., 2001), which had led to prevalence of 37% in populations of up to 120,000 people, with high proportions of sowda (see section 3.3.)

A coordinated interruption of onchocerciasis transmission, which met the criteria set forth by WHO guidelines for interruption of transmission of O. volvulus, has been achieved at the cross-border focus where the Galabat focus adjoins the Ethiopian Metema focus. This success, the first such cross-border initiative in Africa, was accomplished by a combination of annual and semi-annual ivermectin MDA (Katabarwa et al. 2020b).

4.3.1.7.2. South Sudan

About half the population of South Sudan is affected by onchocerciasis with particularly high endemicity in Western Equatoria, and the Northern and Western Bahr el Ghazal areas. CDTI was begun in the mid-1990s and 5,605,726 people were being targeted in 2009 (Lugga and Chane 2011). Recent zones for CDTI were mapped in the context of research on control of nodding syndrome in the country. Pioneering investigations on the biology of the vectors was conducted in South Sudan by Lewis (1953) and by Baker and Abdelnur (1986).
4.3.2. Yemen (Arabian Peninsula)

Yemen is the only country in the Eastern Mediterranean with onchocerciasis, where it is most prevalent along the permanent waterways (wadis) draining into the Red Sea in the west of the country. The vector breeds in very shallow flat wadis with low discharges of up to 1 m$^3$.sec$^{-1}$ (Garms and Kerner, 1982). It is a unique species *S. rasyani*, a member of the *S. damnosum* complex (Garms et al., 1988).

Although planned, to our knowledge, there has been no vector control but sporadic ivermectin distribution was begun in the early 1990s. Since 2011 civil strife and wars have interrupted the national campaign. The disease in Yemen is characterised by high rates of the hyperreactive clinical manifestation known as sowda (Anderson et al., 1973; Büttner et al., 1982; Büttner and Racz, 1983). Mahdy et al. (2018) reported an overall seroprevalence rate of 18.5% during their surveys in 2017. According to WHO (2018a,b) in Yemen in 2017 of about 6.3 million people in 33 districts were requiring MDA.

4.3.3. Americas (OEPA)

In the continent of America onchocerciasis was restricted to six countries of central and south America: Brazil, Colombia, Ecuador, Guatemala, Mexico and Venezuela (Fig. 4). Differing from Africa with its prevalence in large parts of 31 countries, in the Americas the occurrence of onchocerciasis was or is confined to limited foci. Accordingly, the combat against onchocerciasis was different and an elimination of the infection appears to have been or will be feasible by mass drug administration with ivermectin.

A variety of vectors is or was responsible for transmission in central and southern America. Members of the *S. ochraceum* and *S. metallicum* complexes were involved in Mexico, with the former most active in transmission in Guatemala (Garms, 1975b; Rodríguez-Pérez et al., 2015).

A detailed review of the potential importance of further anthropophilic *Simulium* species as vectors of *O. volvulus* in Guatemala has been presented by Takaoka (2015).

In 1993 the Onchocerciasis Elimination Program for the Americas (OEPA), a regional initiative and international partnership, was launched. Sauerbrey et al. (2018) reported on the successful progress toward elimination of onchocerciasis in the Americas. From 1989 to 2016, more than 11 million ivermectin treatments, given twice or four times per year, have been given in the Americas, eliminating transmission in 11 of 13 foci. The number of people at risk of onchocerciasis decreased from >530 thousand to about 30 thousand. Nodulectomy campaigns, e.g. in Mexico and Guatemala, probably helped the success of the MDA (Figueroa Marroquin, 1975).

Onchocerciasis was eliminated in Columbia in 2010, in Guatemala 2011, in Ecuador 2012, in Mexico 2014 and in Venezuela in 2017 apart from a focus in the South. One focus also exists in the north of Brazil.

In the remaining Amazonas focus that straddles the Venezuela-Brazil border the main vectors are members of the *S. oyapockense* and *S. guianense* species complexes and *S. incrustatum* (Shelley et al. 2010).
The OEPA’s success influenced programmes in Africa, especially in Sudan and Uganda, which moved from a control to an elimination strategy in 2006 and 2007, respectively. The successes in the Americas have also influenced WHO guidelines for onchocerciasis transmission elimination. With four of the six originally endemic American countries now having eliminated onchocerciasis transmission, and 95% of ivermectin treatments in the region halted, the regional focus is now on the remaining active transmission zone on the border between Venezuela and Brazil.

5. Future perspective

Recent publications report and discuss the success of elimination or close to elimination of onchocerciasis in limited foci in Africa - in Sudan, Mali, Senegal, Burundi, Chad, Malawi and Nigeria (Tekle et al., 2012; Zarroug et al., 2014, 2016; Walker et al., 2017; Rebollo et al., 2018; Richards et al., 2020). This success is completely or mainly based on MDA, of ivermectin. Exceptions are Bioko, Equatorial Guinea, where vector control was successful, and Uganda where onchocerciasis was eliminated from several isolated foci by combinations of control of the vector S. neavei by ground larviciding with temephos and MDA (Katabarwa et al 2018, 2020a,b; Michael et al., 2020).

The priority is given to treatment with ivermectin. Recently, attention, however, is drawn to the problems of poor coverage and inadequate compliance to MDA (Agyemang et al., 2018; Dissak-Delon et al., 2019). Correspondingly, Verver et al. (2018) discussed why a wide-reaching elimination of onchocerciasis cannot be guaranteed by 2025 and proposed a long-term biannual or quarterly MDA combined with vector control activities as complementary approaches (Routledge et al., 2018) for high-endemicity areas to accelerate progress toward elimination. The proof-of-principle in distinct foci in Mali, Senegal and Sudan indicate a possibility to eliminate onchocerciasis with annual or 6-monthly ivermectin treatment in some endemic foci in Africa. Correspondingly, the published WHO 2030 goals for onchocerciasis were influenced by the cited models (EPIONCHO and ONCHOSIM) on the impact of biannual or quarterly ivermectin treatment frequency and in addition complementary vector control (NTD Modelling Consortium Onchocerciasis Group, 2019; Gates Open Research 2019; Hassan and Shaban, 2020).

Anthelminthic drug alternatives to ivermectin, include the aforementioned Moxidectin (Awadzi et al. 2014, Opoku et al., 2018) and novel anti-Wolbachia agents like the thienopyrimidine/quinazoline scaffold AWZ1066 (Hong et al., 2019).

The timelines of onchocerciasis from control to elimination and eradication were discussed by Kim et al. (2015). They estimated that the elimination scenario will endure until 2028 in all endemic countries except four (Republic of Congo, Central African Republic, South Sudan, Gabon) but CDTI was predicted to continue beyond 2045 in countries with operational challenges, with around 1.15 billion treatments. The elimination of transmission (EOT) of onchocerciasis for the majority of foci in the 34 countries in Africa has been projected in several publications to be between 2025 and 2045 (Dadzie et al., 2018; Gebrezgabiher et al., 2019; Kim et al., 2015).
One major problem is that an elimination of onchocerciasis with ivermectin treatment alone has not so far appeared to be feasible in many African countries where onchocerciasis was endemic over millions of square kilometres spanning more than 30 countries. In addition, the vectors are highly efficient and with much higher endemicity levels migrating over hundreds of kilometres (Dadzie et al., 2003) threatening re-emergence of infected vectors and of onchocerciasis in their wake. Thus, exemplarily, on Bioko where the unique endemic vector (the Bioko form of S. yahense) was rendered extinct (Traore et al. 2009) some vectors may have returned. These are S. squamosum rather than S. yahense (D. Boakye, pers, comm 2019) and it is unknown if they brought any O. volvulus with them.

Nevertheless, given that after nearly 15 years without any transmission on the island very few onchocercal cases remained (Hernández-González et al., 2016; Moya et al., 2016; Herrador et al., 2018; Ta et al., 2018), prospects for confirming elimination are good. The interruption of transmission was considered to have been permanent, but the Bioko case is salutary and illustrates that, however good planning and forecasts can be, there is room for the unexpected to affect our perspectives. In contrast, in the Americas, onchocerciasis elimination with ivermectin treatment has been considered feasible, since most onchocerciasis foci in the Americas were small and circumscribed, and most vector species are relatively inefficient. Thus, interruption of the transmission was feasible by 6-monthly or even 3-monthly ivermectin treatments (Sauerbrey et al., 2018).

Planning MDA programmes is now often based on the outputs of mathematical models such as ONCHOSIM (Plaisier et al 1990) or EPIONCHO (Basáñez et al. 2016) but these models skimped on details of blackfly biology. Only recently have models begun to model vector biology explicitly and started to take account of future uncertainties regarding climate change (Cheke et al., 2015) and the likelihood of needing to supplement MDA with vector control (Routledge et al., 2018). Such vector control in isolated foci could include ground larviciding, slash-and-clear vegetation destruction and killing host-seeking adult female flies in traps such as the Esperanza window trap (Rodriguez-Pérez, 2013; Toé et al., 2014; Hendy et al., 2017; NTD Modelling Consortium Onchocerciasis Group, 2019; NTD Modelling Consortium, Gates Open Research, 2019). These could be supplemented by deploying traps to catch ovipositing female flies such as “Bellec plates” placed beside breeding sites (Bellec, 1976; Cheke et al., 1982).

Difficulties surrounding the elimination of the transmission of onchocerciasis were reviewed by Cheke (2017), who drew attention to cases of successful control where transmission had probably been eliminated in contrast to areas where such interruptions were likely to be only temporary. These included areas in Africa with >55% prevalence, where mass drug administration (MDA) alone was thought of as unlikely to succeed.

In summary, anthelmintic MDA complemented by appropriate vector control measures may increasingly lead to control and hopefully eradication of onchocerciasis which may be fulfilled in mid-century.
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Legends
Figure 1. *Onchocerca volvulus* life cycle: B. Infection: (1.) infective third-stage larva (L3) of *O. volvulus* is transmitted by the vector *Simulium* (A.); (2.) a histological section through an onchocercoma in the human host comprising a coiled adult female developed from a fourth-stage larva; (3.) microfilariae produced by the adult female worm which (4.) migrate from the onchocercoma into the skin (5.) to be taken up by the pool-feeding *Simulium*. (A.) In the vector a microfilaria develops into a first stage "sausage" form larva (L1), moults into the second-stage larva (L2) and then moults again to become a third-stage infective larva (L3) which is transmitted into the skin of the human host when the vector pool feeds (1.) (figure modified from Rudolf Geigy, *Acta Tropica* Supplement 6, Geigy and Herbig, *Erreger und Überträger tropischer Krankheiten*, 1955).
Figure 2. Stages of the *Onchocerca volvulus*: (1.) microfilaria (about 0.2-0.3 mm long), (2.) adult males (1.5-4.5 cm), (3.) infective third-stage larva (0.7-0.8 mm); (4.) adult females (30-60 cm), (5.) an onchocercoma with cut adult females, (6.) sections through a nodule showing adult females (ca. 0.3 mm in diameter) showing two uterus branches with cut microfilariae, cut intestine and lateral cords containing endobacterial *Wolbachia* stained red (photo Brattig et al. 2001; D.W. Büttner, Liberia 1991; N.W. Brattig, BNITM, 2000).
Figure 3. *Simulium damnosum* s.l. stages: (1.) Masses of eggs Niger, Guinea, photo R. Garms, 1963), (2.) larvae on trailing vegetation and (3.) pupae, St. Paul River Liberia (photo R. Garms, 1983). (4.) Adult *Simulium* s.l., photo R. Garms, Liberia, 1983), (5.) Drop of blood after pool-feeding by a *Simulium* (photo R. Gams, Guatemala, 1974).
Figure 4. Prevalence of onchocerciasis (marked red) in 30 sub-saharan African countries, in Yemen (Arabian Peninsula) and in 6 countries of central and south America. The area of the Onchocerciasis Control Programme (1974-2002) is marked yellow (modified, WHO, 1995).
Figure 5. Pathology of onchocerciasis. A. Skin pathology and microfilariae: (1.) depigmentation, (2.) dermatitis, (3.) lichenification and hyperpigmentation (sowda) (Brattig, 2004; photo D.W. Büttner, Liberia 1995), (4.) microfilariae in a histological section of skin (photo D.W. Büttner, Liberia, 1999), (5.) microfilaria released in vitro from a skin snip (photo N.W. Brattig, 2000); B. Onchocercomata (6.) nodules in the torso of a man (photo D.W. Büttner, Liberia, 1985), (7.) nodule in the leg of a boy (photo R. Garms, Liberia, 1971), (8.) A bisected onchocercoma with adult females (Brattig, 2004).
Figure 6. Eye pathology (River blindness). (1.) Two onchocercomata in the forehead of a young child which can release microfilariae in the close proximity of the eyes (DW Büttner, Liberia, 1993), (2.) cornea opacification, sclerosing keratitis (R. Garms, Kouroussa, Guinea, 1963), (3.) man blinded by onchocerciasis guided by a child (photo R. Garms, Sérékoroba Guinea, 1963). Microfilariae releasing the onchocercoma can infiltrate the eye and can lead to visual impairment and blindness.
Figure 7. Host-parasite interaction. (1, 2.) time-dependent *in vitro* attack of microfilariae (Mf) by human eosinophilic effector cells (3, 4.) and of infective 3rd stage larvae (L3) (modified figures from Brattig, 2004), (5.) Host neutrophilic granulocytes assembled at the surface of a female in a section of an onchocercoma with *Wolbachia* in the lateral cords of the female; the *Wolbachia* were stained with antibodies against *Wolbachia* heat shock protein 60, the neutrophils with antibodies against defensin (Brattig et al., 2001).
Figure 8. Container with Ivermectin (dihydro-avermectin), the principal drug against onchocerciasis (1);
(2) the chemical formula: a macrocyclic lactone, synthesized by Streptomyces sp., administered as
Mectizan® (Merck Inc.) is donated free to millions of onchocerciasis patients organized by the Carter
Center. (3) In 2015, the Nobel Prize in Physiology or Medicine was awarded at the Karolinska Institute to
William C. Campbell and Satoshi Ōmura for their discovery of the anti-filarial compound Ivermectin.
Figure 9. Antibacterial doxycycline, (1.) a tetracycline-class polycyclic antibiotic agent kills *Wolbachia* endobacteria in *Onchocerca* microfilariae and is introduced for treatment of onchocerciasis in 2003 (Hoerauf et al., 2001; Walker et al., 2015). (2.) Sections through onchocercomata (A.) from an untreated patient which comprise *Wolbachia* stained red in the cords and (B.) females in an onchocercoma from doxycycline-treated patients with depleted *Wolbachia* (Brattig et al., 2001).
Figure 10. Progress of onchocerciasis elimination in Uganda by 2019. Comparison of transmission in 1992 (1.) and in 2019 (2.) In 1992 (1.), only the historical large Victoria Nile focus (green) had been eliminated after intensive vector control activities. In 2019 (2.), onchocerciasis had been very successfully eliminated from 15 (green) of the original 17 foci, transmission was possible still ongoing in the Madi Mid North focus (focus 17; light green) and still active in the small Lhubiriha focus on the border with D.R. Congo (focus 14; red) (copyright MOH Uganda and The Carter Center; 2019 kindly provided by Moses Katabarwa, 2020).