Arbonematodes – Nematode Infections Transmissible by Arthropods

Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger»*

Introduction

Some alphaviruses and flaviviruses, such as the transfusion-relevant Chikungunya and West-Nile virus, are transmitted by mosquitoes. Such viruses are called arboviruses. Bacteria typically transmitted by ticks include *Coxiella burnetii* [1], Borrelia, Ehrlichia, and Anaplasma. These are referred to as arbobacteria [2]. Protozoae include plasmodia which are transmitted via mosquitoes, leishmania transmitted via sand flies (Phlebotomus), and for example trypanosoma transmitted via kissing bugs (Triatoma). The latter are grouped under the name arboprotozoa [3]. The following nematodes are transmitted by arthropods as vectors to humans: microfilaria of *Wuchereria bancrofti* via mosquitoes (Culex), Onchocera (*Onchocera volvulus* in Africa, *O. caecutiens* in South America) via black fly (Simulium) and *Loa loa* (African eye worm) via horse flies (Tabanidae) – therefore these agents are here grouped under the name arbonematodes.

1. Current Knowledge about the Pathogen

Helminths (worms) are subdivided into nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms). Among nematodes, a distinction is made between intestinal and tissue roundworms. Intestinal nematodes are subdivided into the filarial worms, such as *Ankylostoma duodenale, Ascaris lumbricoides* and *Strongyloides stercoralis*, which pass through the lung during maturation and re-enter the intestine thereafter; they only have a short hematogenic phase. In the case of trematodes, the larvae of schistosoma (*Schistosoma mansoni, S. haematobium* and *S. japonicum*) live only in the portal vein. Like intestinal nematodes, they achieve maturity by passing through the lung, and thus their larvae can also be present in the blood for a short period. The worms migrate through the veins to th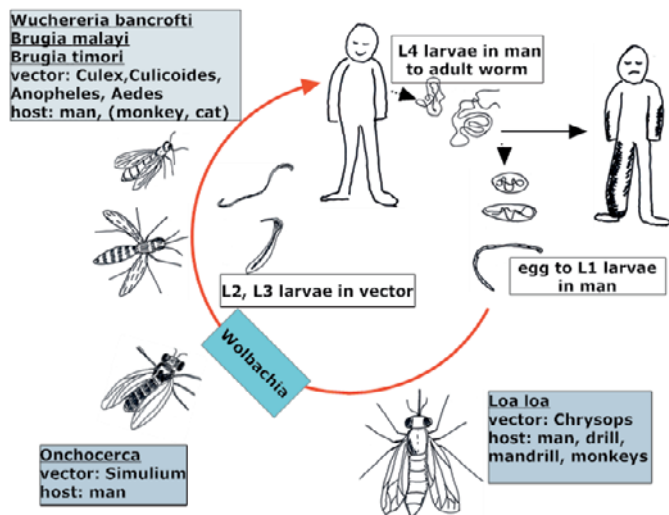e bladder and rectum at night time where they deposit their eggs in the tissue after penetrating the wall of the blood vessel. The eggs are excreted with urine and feces. The larvae which develop from the eggs are ingested with food or water or acquired via skin passage, and do not fall into the category of arbonematodes. They are not transfusion-relevant. Therefore this article will not provide more detailed information on this species [4].

Tissue roundworms include *Trichinella spiralis, Dracunculus medinensis* and *Toxocara canis*, which remain within tissues and neither release eggs nor microfilariae into the blood; they are not transmitted via arthropods. Nematodes which are transmitted to humans via arthropods include lymphatic filaria such as *W. bancrofti, Brugia malayi* and *B. timori*, as well as Mansonella, Onchocerca and *Loa loa* which live in tissues.

1.1. Characteristics

1.1.1 Life Cycle

Arbonematodes are hermaphroditic worms with a reproduction cycle in humans or primates and a maturation cycle of the larva in the appropriate arthropods (fig. 1). After the sting of a female mosquito or horse fly, who need blood for the maturation of their eggs, the L3 larvae leave the salivary gland of the arthropod and pass from the surface of the skin through the penetration canal into the subcutis [5]. From there, they pass into the lymphatic vessels, develop into L4 larvae, and, after another 6–9 months, develop into sexually mature male and female worms. The worms stay in the afferent lymphatic vessels of the extremities and of the male genital and in the skin (fig. 1). The fertilized female worm releases around 10–20,000 eggs daily into the lymphatic system or blood stream. The L1 larvae, called microfilariae, hatch from these eggs. If an infected human is stung by a mosquito or horse fly, the L1 larvae are consumed with the blood meal, penetrate through

Prof. Dr. med. Rainer Seitz
Paul-Ehrlich-Institut
Paul-Ehrlich-Straße 51–59, 63225 Langen, Germany
Rainer.Seitz@pei.de

Fig. 1. Schematic drawing of the replication cycle of arbonematodes in the vector (horse fly, black fly, Anopheles, and Culicoides) and the human host. Adult female worms produce > 10,000 eggs per day from which microfilariae develop into L1 larvae (dotted arrow). Further maturation into L3 larvae takes place in the vector (red arrow with continuous line) – only for *Loa loa* without involvement of Wolbachia bacteria. The vector transmits L3 larvae to humans during stinging. These L3 mature into L4 larvae and develop into adult worms. The male worm is smaller than the female (schematic drawing by Guertler).

the intestinal wall of the arthropod, and develop into L2 and L3 larvae in the thorax muscles after 2 weeks [6]. The L3 larva migrates into the head of the arthropod, then to the region of the masticatory apparatus, and is deposited on the human skin during the stinging process. Reproduction of the larvae in the arthropod does not take place. The death rate of the L3 larvae is very low [5].

1.1.2 Lymphatic Filariae

Wuchereria bancrofti
The adult worm with a length of around 4–8 cm (female > male) resides in the lymphatic vessels of the host, which are partially mechanically obliterated and dilated by inflammatory processes. The worm is able to survive 5–8 years, and during this period releases millions of eggs and microfilariae (L1 stage), approximately 10,000 daily, with a length of 100–150 μm, into the lymphatic system and the blood [5]. The highest release of microfilariae takes place at night during the stinging period of the mosquitoes between around 10:00 p.m. and 4:00 a.m. [6, 7]. The microfilariae are ingested by the mosquitoes, mature in the latter to the L3 stage, and can then be transmitted again during the blood sucking process. Many mosquito species, such as Anopheles, Culex, Aedes, Mansonia and Culicoides, are capable of transmitting microfilariae. Culex species spread *W. bancrofti* preferably in Africa, and Aedes is preferentially involved in the transmission of *B. malayi* [8]. The worm is colonized by the bacterial species Wol-

bachia. Wolbachia is essential for larva development and sex determination. If Wolbachia is missing, for instance due to treatment with tetracycline, the male to female ratio of the larvae changes, displaying a preference of male larvae since female larvae require a denser population of Wolbachia for differentiation [9]. Without a Wolbachia colonization, the adult worm of *W. bancrofti* can survive only 12 months [7].

Brugia malayi and Brugia timori
Morphologically, the adult worm is very similar to *W. bancrofti*. The microfilaria of *B. timori*, however, is clearly larger, the sheath cannot be stained by Giemsa staining, and 2 nuclei are positioned at the posterior end, while those of *W. bancrofti* and *B. malayi* are located in the tail of the larva [6]. The endosymbiont Wolbachia is essential for the development of the larva and the survival of the worm.

Loa loa (African eye worm)
Transmission occurs by diurnal horse flies of the Chrysops species. The adult worm is 3–7 cm in length and develops within 6–12 months from the larva. The worm lives in the skin, and occasionally also occurs in the eye. The eggs are released during the daytime by the mature female worm and develop into the L1 larva. Further development into L2 and L3 occurs in the horse fly after ingestion of the microfilariae during the blood meal (fig. 1). The microfilariae have sheaths which they shed during maturation in the horse fly. The adult worm lives in humans for 4–12 years whereas microfilariae survive 6–12 months [10]. The endosymbiont Wolbachia is missing within *L. loa* and is not necessary for worm development. Drill and mandrill (*Mandrillus leucophaeus*, *M. sphinx*) are the major non-human hosts; furthermore *L. loa* is endemic in West African and Central African Putty-nosed monkeys/great spot nosed monkey/white nose monkey (*Cercopithecus nictitans*) and Mona monkeys (*Cercopithecus mona*).

Mansonella
Mansonella perstans is transmitted by Culicoides. The adult female worm is approximately 7–8 cm in length, the male approximately 4–5 cm in length. The worm prefers serous cavities of the body and releases its eggs or microfilariae, which are equipped with head sheaths. *M. perstans* occurs in Central and North Africa and in the Caribbean. *M. ozzardi* is transmitted by Culicoides and *Simulium amazonicum* in South and Central America and in the Caribbean. The microfilariae do not bear a head sheath. *M. streptocerca* is transmitted by Culicoides. The worm is endemic in the rain forests of East and Central Africa. The microfilariae preferably live in the skin and can be identified by skin biopsy (skin punch, skin snip) by their fish hook-shaped tail.

Onchocerca
The worms and the mircofilariae live mainly in the subcutis. Thousands of microfilariae without sheaths are released daily

by the adult female worm (size around 40 × 0.03 cm). The male worm is smaller (around 3 × 0.03 cm) and survives in subcutaneous nodules which are easily palpable [5, 7]. The microfilariae survive in humans for 12–18 months. When they die, they cause an itchy chronic inflammation due to the release of Wolbachia. The microfilariae are 200 μm in length; if the eye is affected, they can be detected directly or using a slit lamp. Wolbachia maintains and accelerates inflammatory processes by chemokine production, which leads to infiltration with neutrophil granulocytes at the dying site of the microfilariae. Furthermore, immune suppression and T-helper lymphocyte-dependent Th2 response is caused by endosymbiosis with Wolbachia. Onchocera from savannah regions, where river blindness (onchocercosis) is wide-spread and endemic, are infested with Wolbachia to a considerably higher extent than in other regions so that the presence of the bacteria appears to play an essential role for the manifestation of blindness.

Dirofilaria repens
Dirofilaria repens is a parasite which infects dogs and cats. In the eyes of humans, the filariae undergo an incomplete development [5]. In most cases, they remain in the human skin. Dirofilaria also occurs in Southern Europe, clusters of high frequency have been described in some regions of Serbia [11]. Humans are accidental hosts. Therefore, dirofilaria will not be described in more detail.

Setaria labiatopapillosa, S digitata
These microfilariae occur in domestic animals in East Asia and Southern Europe. They are occasionally transmitted to humans where they cause subconjunctival inflammation. Encephalomyelitis caused by Setaria has been described [12]. Setaria has no significance for transfusion medicine.

Vectors
In rural regions, particularly Anopheles mosquitos transmit microfilariae. Species include *Anopheles gambiae*, *Anopheles funestus*, *Anopheles punctulatus*, and 40 other species. Species that occur in urban regions include *Culex quinquefasciatus*, *Culex pipiens*, and further species, as well as *Aedes polynesiensis* and Mansonia and Ochlerotatus [8, 13].

Among horse flies (Tabanidae), the species that transmits parasites belong to the genus Chrysops, in particular *Chrysops silacea* and *Chrysops imidiata*, which are diurnal and anthropophilic, furthermore *Chrysops distinctipennis*, *Chrysops zahrai*, *Chrysops centurionis* and *Chrysops longicornis*, depending on the region [10].

The development of Simulium larvae is dependent on oxygen-rich water; therefore the black fly is found in the vicinity of small waterfalls and rapids. Such regions include the gallery forests in savannahs, in particular in West Africa and South America [14]. The black fly and Onchocerca are adapted to humans.

1.2. Infection and Infectious Disease

1.2.1 Pathogenesis

Wuchereria bancrofti, Brugia malayi and B. timori
Typical symptoms in humans do not develop before the age of 10 years and only in a minority of infected individuals, suggesting that in addition to immunological factors, genetic factors are involved in the manifestation of the disease [15, 16, 7]. The infection always takes a chronic course. Co-infections such as malaria and tuberculosis exacerbate immunosuppressive effects caused by filariae [17]. The filaria infection reduces Th1 and Th17 lymphocyte actions against *Mycobacterium tuberculosis* [17]. In addition, a part of the pathogenesis is due to the Wolbachia released by the dying worm. Components of Wolbachia cause activation of macrophages and complement, and attract neutrophil granulocytes. The worm is surrounded by an inflammatory granuloma. Th1 and Th17 helper lymphocytes are activated by Wolbachia components [7]. Nematode components induce a Th2 response with allergic reactions such as eosinophilia and IgE production. After years of infection, damage to the lymphatic vessels will lead to hydrocele and lymph edema with elephantiasis. The symptoms, especially on the skin, are exacerbated by opportunistic infectious agents. Increased levels of vascular endothelial growth factor-A (VEGF-A) are associated with the manifestation of the hydrocele, and VEGF-C is associated with lymph edema. In Ghana, homozygosity of C/C in position 260 of VEGF-A is associated with the frequency of the hydrocele [15]. Treatment with tetracycline will reduce VEGF-A levels, which indicates that VEGF-A production is induced by Wolbachia. Blood coagulation is impaired in patients with chronic microfilaria infection [18].
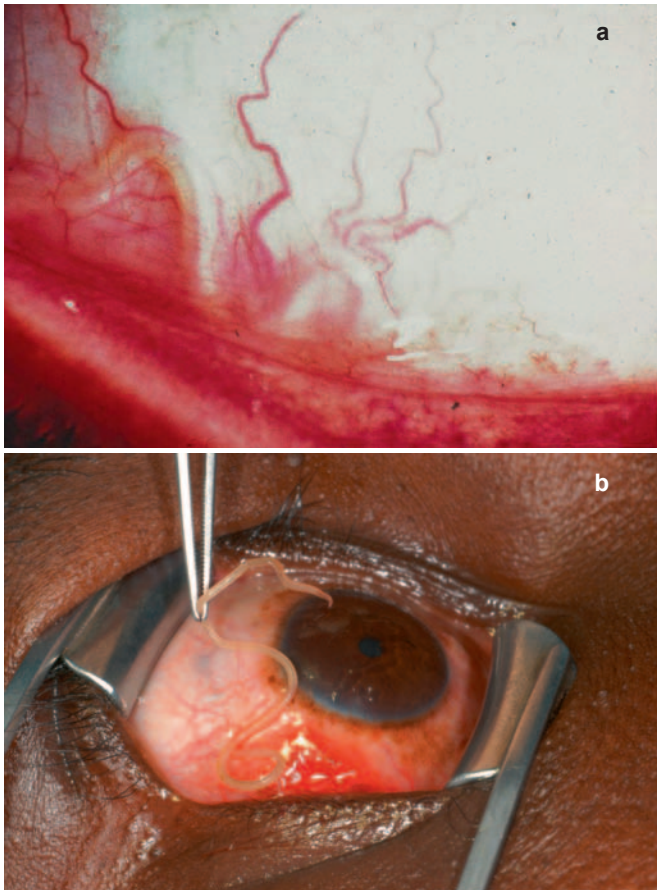
Loa loa
After transmission of *L. loa* by horse fly sting, the first symptoms occur after 5 months, but clinical latency can persist up to 13 years. In the case of colonization with low numbers of worms and in children, the infection is often asymptomatic. If the eyes are infected, a worm takes around 30–60 min, in some cases up to a day, to enter the eye (figs. 2 a and b). Invasion is accompanied by photophobia, itching, pain, and swelling. Overheating of the eye may induce migration of the worm. Finally lytic processes in the eye may lead to blindness. Calabar swelling, a short-term, painless, and partly itchy edema mostly of the forearm, is caused by a subcutaneous allergic reaction to *L. loa*, which occurs in particular in migrants in endemic regions. Calabar swelling is accompanied by eosinophilia [10].
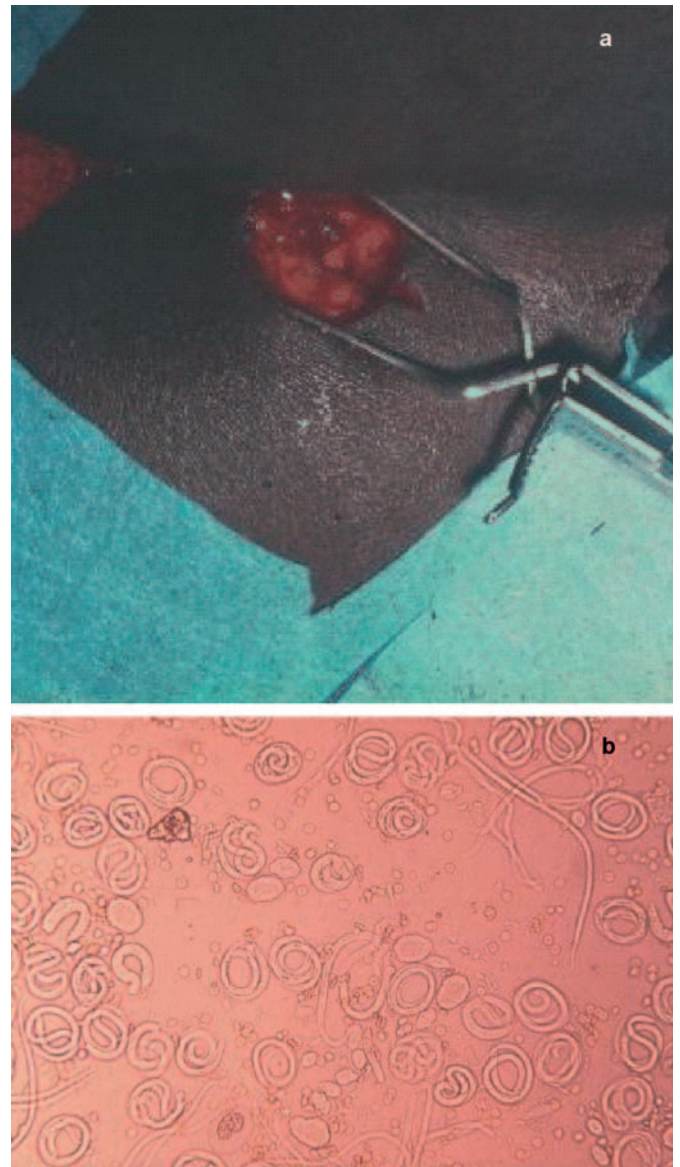
Onchocerca
After transmission, pathogenic damage initially occurs preferentially in the skin. Thousands of microfilariae per day are released from the worm convolute (figs. 3 a and b) which

Fig. 2. *Loa loa* infection of the eye. **a** Convoluted microfilaria in the conjunctival sac. **b** Microfilaria held by a pair of tweezers after extraction (photo: Prof. Volker Klaus, Ophthalmological Hospital, University of Munich).

Fig. 3. *Onchocerca volvulus*. **a** Convolute and preparation of the worms. Worms are surrounded by connective tissue. **b** Microfilariae which migrated from a skin biopsy (skin snip), on a microscopic slide in sodium chloride. The length of the microfilariae is around 250–300 μm (photo: Prof. Thomas Löscher, Department of Tropical Medicine, University of Munich).

usually consists of several male and female worms. The microfilariae cause a local inflammation which manifests itself as a continued itchy irritation, acute and chronic papular dermatitis, and lichenoid 'oncho-dermatitis' (scleroderma, erysipelas). The skin can be affected by depigmentation (so-called leopard skin), and the elasticity of the skin may be reduced – these changes are triggered by a hyperimmune response (Th2 response) [19, 7]. African onchocercosis (*O. volvulus*) manifests on the torso, while the American variant (*O. caecutiens*) manifests on the head. Onchocerca microfilariae display a high affinity for the eye. After migration to the eye, some die at the entry site, and cause a severe inflammatory reaction with keratitis and irodocyclitis, finally retinitis, and damage to the optical nerve, which causes visual impairment and finally blindness (→ river blindness). The ocular inflammatory reaction is exacerbated by Wolbachia components. Migration from neutrophil granulocytes will lead to opaqueness of the cornea [7]. The daily turnover, the number of dead and newly produced microfilariae, is 10,000–300,000; up to 150 million microfilariae can be present in the body [20].

1.3. Epidemiology

Since microfilariae are transmitted by vectors, their geographical distribution is closely related to the habitats of the responsible vector. Worldwide, around 110 million people are affected by *W. bancrofti*, around 10 million by Brugia, around 37 million by Onchocerca, around 10 million by *L. loa*, and less than 10 million by Mansonella. The different microfilariae prevail in the following endemic regions (fig. 4):

Wuchereria bancrofti: Africa, south of the Sahara, South-East Asia, Oceania, the Caribbean, and restricted regions in South America. Countries with hyperendemic prevalence in-

Fig. 4. Rough display of the world-wide distribution of arbonematodes by country. Endemic regions of *Wuchereria bancrofti* and Brugia are marked in yellow ochre, those of Onchocerca in light blue with horizontal lines and overlapping regions in purple. Additional *L. loa* infections are limited to a belt in West and Central Africa. They are marked in yellow and with a hatched pattern (diagram: Guertler).
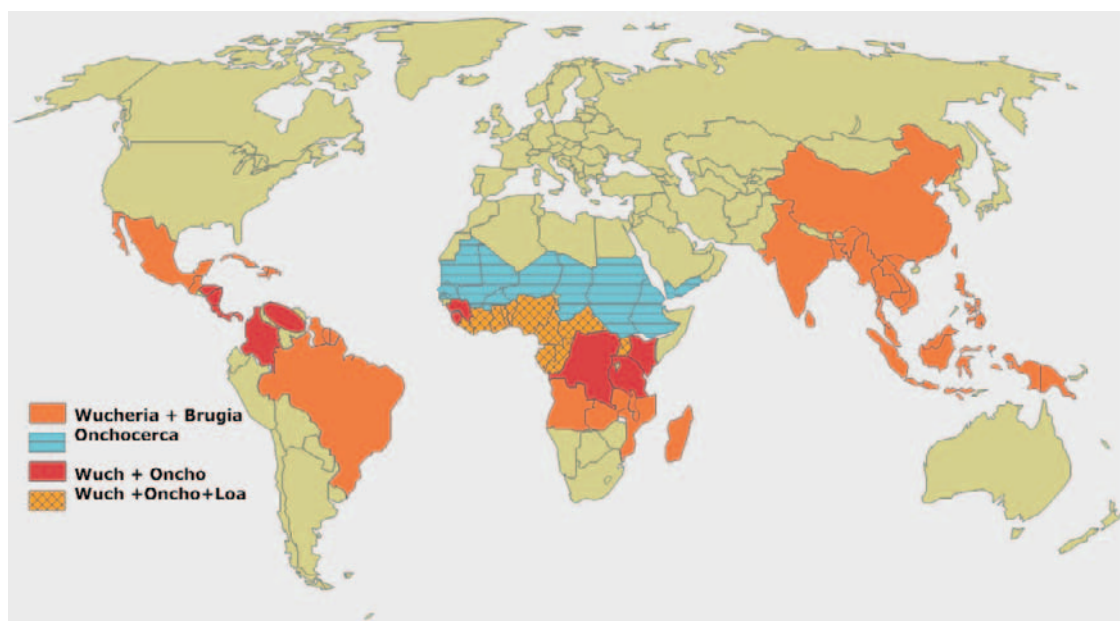
clude India, Indonesia, New-Guinea, Nigeria, Ghana, Kenya, and Tanzania. Vectors are Anopheles which are twilight-active, and the nocturnal species of Culex and Aedes mosquitos. Up to now, no animal reservoir is known.

Brugia malayi: Southern Asia and South-East Asia as well as the Pacific region. In addition to humans, cats and primates are a reservoir. Vectors are similar to *W. bancrofti.*

Brugia timori: New-Guinea and the islands of East Indonesia. No animal reservoir is known. Vectors are similar to *W. bancrofti.*

Loa loa: West and Central Africa up to Uganda and Ethiopia. Primates are a reservoir, especially drill (*M. leucophaeus*) and mandrill (*M. sphinx*) [10]. A case of *L. loa* infection of the eyelid was reported in an African student in Germany [21]. Vectors are the diurnal *C. silacea*, *C. dimidiata*, and other *Chrysops* species.

Mansonella: M. perstans – Africa, south of the Sahara, North-Africa, northern coast of South America; *M. ozzardi* – Caribbean, Central and South America; *M. streptocerca* – West and Central Africa. Culicoides species and *Simulium amazonicum* are vectors for *M. ozzardi*, Culicoides for *M. perstans* and *M. streptocerca.*

Onchocerca: Africa south of the Sahara, in particular the savannah regions of Nigeria up to the Congo and their gallery forests are affected; Middle East and Southwest Asia, Central and South America with low prevalence and a low number of worms [5]. In hyperendemic regions, more than 60% of the population host filariae in the skin. Vectors are: the black fly – *S. damnosum* – in West Africa, *S. naevei* in East Africa, *S. ochraceum*, and *S. metallicum* in Central and South America. An animal reservoir for *O. volvulus* has so far not been described. Animal worms are *O. gibsoni*, *O. gutturosa*, and *O. reticularis.*

The current epidemiology of arbonematodes is significantly influenced by treatment using a combination of diethylcarbamazines or ivermectin with albendazole or mebendazole, which significantly reduces filaremia in the human host. An investigation of blood samples taken by night for *W. bancrofti* in the north of Malawi in 2000/2001 showed filaremia in 21%, hydrocele in 12%, and lymphedema in 1% of the men, and 4% of the women in [22]. From 2000 to 2003, guest workers (> 90% from India) in Kuwait were tested for lymphatic filarie: By using an antigen test, 18.3% were found to be positive; whereas by microscopy 3% were found to be carriers of filariae (median value 816 filariae/ml), while none of these individuals had malaria [23]. Samples from patients from high-risk countries were tested in the USA from 1999 to 2009 using conventional parasitological methods including blood filtration, as well as nucleic acid testing (NAT) by real-time polymerase chain reaction (PCR). For *W. bancrofti* a prevalence of around 4% was found using PCR; for *L. loa*, this value was 11%. Conventional microscopy of the skin biopsy (skin snip) to detect *O. volvulus* resulted in a prevalence of around 1%, but PCR revealed a value of 6%.

The simultaneous administration of diethylcarbamazine and albendazole for the treatment of *W. bancrofti* infection in a study in Brazil did not result in a significant reduction of the microfilaria number and the worm nests after 1 year [24]. Administering diethylcarbamazine 10 times led to a reduction in *W. bancrofti* microfilariae by over 90% in India, while administering ivermectin 9 times resulted in a reduction by around 85% [25]. The combination of ivermectin and albendazole was more efficient against *W. bancrofti* microfilariae, even though in other studies, the combination of diethylcarbamazine was superior [26]. In another analysis from Sri Lanka, the administration of diethylcarbamazine was superior

since ivermectin is highly active against microfilariae while it has only very low activity against macrofilariae [27]. Doxycycline reduces infestation with microfilariae [27]; the same applies to rifampicin [8].

A single administration of ivermectin per year led to a reduction in the prevalence of microfilariae of Onchocerca from 70 to 40% in Nigeria after 6 years [28]; in Cameroon and Uganda this figure was reduced from 70 to 7% after 10 years [29], and in Equatorial Guinea from 75 to 38% after 8 years, with significant differences between 2 ethnic tribes and individual communities [30]. Another study from Ghana reported that the prevalence of Onchocerca microfilariae doubled in 2 communities while the general infestation with microfilariae was reduced. This phenomenon was caused by resistance against ivermectin [31]. Age, gender, and hyperendemic prevalence of Onchocerca are other factors that influence the efficacy of ivermectin treatment [16].

In India, the blood of 119 pregnant women was tested for the presence of microfilariae of *W. bancrofti* and their antigen (Og4C3), as well as the cord blood of their newborns: microfilariae were found in 12% and antigen in 44.5% of the samples of maternal blood while only 24.5% of the newborn babies of the microfilaria antigen-positive mothers were antigen-positive. It was concluded that the diaplacental antigen transfer was responsible for early sensitization of the children [32].

Notification Requirement
The detection of or infection with arbonematodes is not notifiable pursuant to the Infection Protection Act (Infektionsschutz-Gesetz).

1.4. Detection Methods and their Significance

### 1.4.1 Detection by Microscopy
Around 1.5 years elapse before the adult worm produces a sufficient amount of microfilariae to be detected in a preparation under the microscope [20].

Native preparation: By centrifugation of anticoagulated venous blood, microfilariae accumulate at the layer between the red blood cells and the plasma (buffy coat). Motile microfilariae can be recognized easily under the microscope (fig. 5); differentiation of the microfilariae is not possible using this method [33].

Detection in a thick blood smear: After staining with hematoxyline-eosine solution of the lysed red blood cell drops, microfilariae can be detected but barely differentiated.

Concentration by centrifugation: After lysing the red blood cells, preparing a smear of the sediment and staining, or after density gradient centrifugation [34], microfilariae can be concentrated from larger quantities of blood.

Detection by filtration of blood after lysing the red blood cells using a 5 μm polycarbonate filter and subsequent stain-
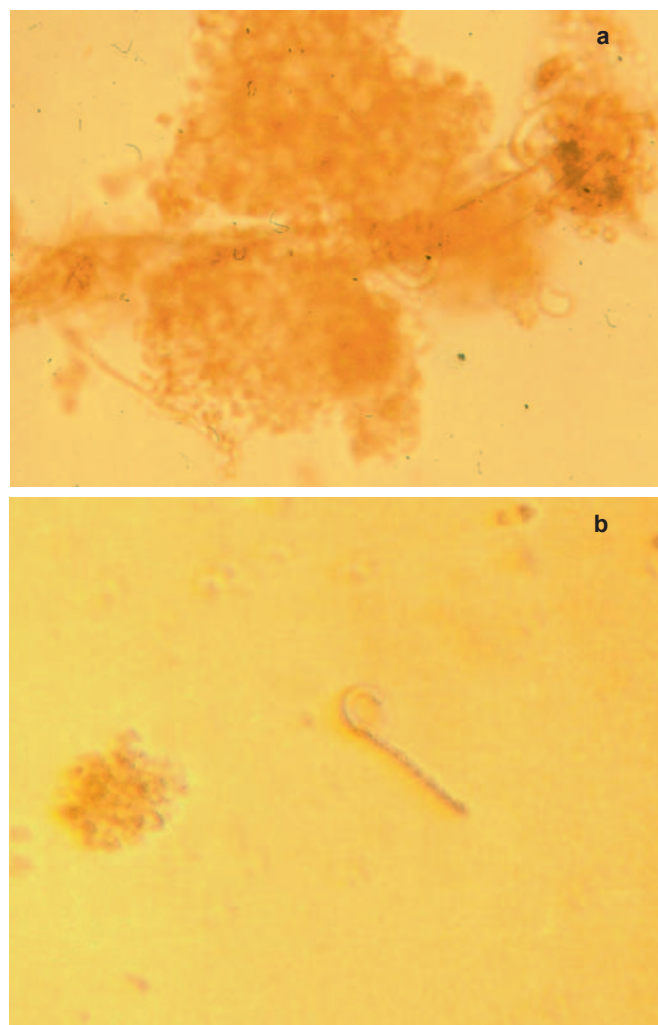
Fig. 5. a 5 microfilariae, partly adhering to a cotton fiber, in the native culture after enrichment by means of Ficoll-Paque gradient centrifugation. The cell clusters consist of lymphocytes and macrophages with a single cell mean diameter of 7 μm. **b** Singular microfilaria in the center and left of this microfilaria lymphocyte aggregate (compound and photo: Guertler).

ing [33]: Microfilariae are around 150–300 μm in size. *W. bancrofti*: The microfilaria has a sheath; the tail end does not contain any nuclei; *B. malayi*: The microfilaria has a sheath; the tail end contains 1–3 small nuclei; *L. loa*: The microfilaria has a sheath; the tail end has several nuclei; *O. volvulus*: The microfilaria has no sheath; the tail end is of pointed conical shape and has no nuclei; *M. perstans*: The microfilaria is smaller (about 200 μm), the tail end is rounded (fish hook) and contains nuclei [33].

Skin biopsy (skin snip) and fine needle puncture: In severely itchy areas, the skin is punctured, and the biopsate is placed under the microscope in saline solution on a slide. After a short period, the microfilariae, in particular those of Onchocerca, evade the biopsate and can then be detected by their movement.

1.4.2 Antibody Detection

A rapid test for the detection of Onchocerca IgG4 antibodies which contains glutathion-S-transferase (Ov16) as antigen showed a sensitivity of 91% and a specificity of 95% during the examination of 106 infected patients. Cross reactivity exists with other filarial infections [35]. Based on 4 antigens of *O. volvulus* using a luciferase immunoprecipitation system (LIPS), 100% sensitivity and 100% specificity was found in Onchocerca infected individuals [36]. A test which was confected in a similar way for the detection of *L. loa* antibodies reached a sensitivity of 67% and a specificity of 99%. Cross reactions existed with *O. volvulus*, *M. perstans*, and *W. bancrofti* [37]. A chromatographic dipstick assay for the detection of an infection with Brugia showed a sensitivity of between 61 and 97% and a specificity of 100%. It showed a specificity level for *O. volvulus* of 99%, and for *L. loa* of 85% [38].

1.4.3 Antigen Detection

An Og4C3 assay from dried finger prick blood for the detection of *W. bancrofti* antigen showed 100% sensitivity and 94% specificity, and was thus superior to microscopy [39]. With the aid of the Og4C3 antigen ELISA using nocturnal blood collection as indicator for the infestation with adult worms among children in India, Bal et al. [40] found a prevalence of 32%, while with the aid of microscopy the detection rate for microfilariemia was only 10%. Rocha et al. [41] compared the sensitivity of 2 antigen tests (Og4C3-ELISA and AD12-ICT card test) for circulating antigen of *W. bancrofti* (samples collected at night time) with the ultrasound analysis for the detection of the infestation with adult worms. A sensitivity rate of 97% was reached by means of the antigen test and 73% with the aid of the ultrasound method.

1.4.4 Genome Detection (NAT)

The most sensitive method of detecting arbonematodes in the human blood, tissue, or vector is the amplification of nematode nucleic acid. The aim is to detect 1 microfilaria per ml. As genomic region, the Ssp1 DNA repeat sequence of *W. bancrofti* was amplified [42, 43], and a sensitivity rate of 95% at a specificity rate of 100% was reached in blood samples drawn at night time (100 μl). For samples drawn during the day, the sensitivity was lower, accounting for 70% [43].

After amplification of a 320 bp sequence (HhaI repeat DNA) of *B. malayi* via real-time PCR (Taqman PCR) or the minor groove binder (MGB) technology, 22,000 copies of repeat DNA were detected by means of Taqman PCR and 22 copies by means of the MGB test. Both detection methods were negative for *B. pahangi* (occurring in monkeys), and *W. bancrofti* microfilariae [44]. A one-step multiplex PCR for the simultaneous detection of *B. malayi* and *W. bancrofti* Ssp I repeat DNA was developed by Mishra et al. [45]. The test was more sensitive than microscopy and reached a sensitivity rate

of 1 microfilaria per 100 μl blood. A nested PCR allowed the distinction between infections with filariae from *O. volvulus*, *M. ozzardi*, and *M. perstans* [46].

A multiplex real-time PCR for the simultaneous detection of *W. bancrofti*, *Plasmodium falciparum* and *Plasmodium vivax* DNA in Anopheles mosquitoes was developed for epidemiological investigations [47]. An additional method for the simultaneous detection of *P. berghei* und *Dirofilaria immitis* via LAMP amplification (loop-mediated isothermal amplification) was described in 2010. This method is capable of detecting 100 plasmodia and 1 dirofilariae in the starting material, usually blood [48].

After enrichment of microfilariae by filtration of 1 ml blood and drying of the filters, *W. bancrofti* was detected by PCR of the Ssp I repeat sequence and Brugia by PCR of the Hha I repeat sequence. The prefiltration increased the sensitivity of the method [49]. If 5–10 dried filters were pooled, the sensitivity reached was still sufficient. PCR outclasses microscopy in sensitivity even in experienced laboratories [50].

2. Blood and Plasma Donors

2.1 Prevalence and Incidence in Donor Populations

No information on the prevalence and incidence of microfilariosis is currently available for Germany. Tourists, who spent longer periods of time in endemic areas, can be carriers of microfilariae due to a mosquito or a horse fly bite after a period of 6 months or longer which is needed for development of the adult worm. Development and pathogenesis are described in section 1.2. The probability of an individual to develop a carrier status is less than 2% [51]. Among Spanish travelers returning from tropical countries south of the Sahara, 1.7% were infected with filariae [51]. In 1978, a *M. ozzardi* infection was detected in a repeat blood donor in the USA. The donor had acquired the infection during a journey to the Caribbean. The risk of acquiring microfilariae by travelling to an endemic area is estimated to be 1 in 500 by the authors [52]. A filaria infection was detected in 271 (0.63%) of 44,000 travelers who returned to their home countries, and was considerably high if they had spent more than 1 month in the endemic tropical areas. Out of these 271 examined individuals, 37% harbored Onchocerca, 25% *L. loa*, 25% *W. bancrofti*, and the rest was infected with other filaria species [53]. According to the Institute for Tropical Medicine of the University of Munich, microfilariae are annually detected at that institute in around 20 individuals who are immigrants and persons returning from West Africa (Prof. Dieter Notdurft, personal communication). After the end of World War II, no cases of transfusion-acquired microfilaria infections were reported in Germany. In the USA, only 1 case was reported [54].

2.2 Definition of Exclusion Criteria

The deferral period of 6 months for individuals returning from tropical regions because of malaria infections is not a sufficient exclusion criterion for a possible transmission of microfilariae, since filariemia may develop with even longer delay (cf. 1.2). Typical symptoms of infestation include itching and intermittent visual impairments. Donors with the respective travelling history and symptoms as described above should be deferred from donating blood until a laboratory analysis has ruled out an infestation with microfilariae.

2.3 Donor Testing and Significance

Donor testing for microfilariae by a sensitive PCR is possible, however, is not justified because of the low incidence.

2.4 Donor Interviews

A specific donor interview concerning the history of travelling takes place, but an interview on the risk of infection with filariae does not take place and is not required based on the low prevalence and incidence.

2.5 Donor Information and Counseling

Donor information and counseling is available from the Institutes of Tropical Medicine and other centers for infectious diseases and specialized outpatient wards for tropical medicine.

3. Recipient

3.1 Prevalence and Incidence of Blood-Associated Infections and Infectious Diseases in Recipient Populations

No publications are available on the prevalence and incidence for Germany. As has been mentioned above about individuals returning from endemic regions (cf. 2.1), recipients can also be infected with filariae after staying in endemic regions before a blood transfusion.

3.2 Immune Status (Resistance, Existing Immunity, Immune Response, Age, Exogenous Factors)

Microfilariae can be acquired through a sting of the infected vector at any age. Low quantities of L1 microfilariae, which may be present in a blood component and which may then be transmitted, are removed from the blood stream by the response of natural immunity (innate immunity) [54].

3.3 Severity and Course of the Disease

Intravenous (i.v.) transmission of L1 microfilariae to humans (self-test by Gönnert and artificial infection of patients) lead 16–24 h after transmission to fatigue, faintness, dizziness, headache, and rise in temperature to up to > 39 °C for a few days; the febrile reaction was accompanied by eosinophilia [55, 56]. While the L1 microfilariae of *W. bancrofti* were removed from the blood within only 4–5 days [56], the L1 microfilariae of *M. perstans* remained in the recipient for up to 37 months without causing any symptoms [55]. In another study, *W. bancrofti* microfilariae were no longer detectable in humans after 12–18 days after i.v. injection. After i.v. injection into mice, they were eliminated from the blood stream after only 40 h; after intraperitoneal and subcutaneous injection into mice or rats, they survived for 14 days [57].

Years may pass after transmission of L3 larvae of filariae by the vector to humans before typical symptoms become evident (cf. 1.2 Pathogenesis). As one of the first symptoms allergic reactions occur which manifest themselves particularly as itching and partly severe pain if the eyes are affected. Worm clusters develop in the skin, which are visible and palpable; after a number of years, an inflammation occurs with dilation of the lymphatic vessels together with subsequent elephantiasis. When the adult worms die, this can cause strong inflammatory reactions due to the release of Wolbachia (see above 1.2). The disease is curable after several treatment cycles with doxycycline and diethylcarbamazine or ivermectin and albendozol or mebendazol (cf. 3.4).

3.4 Therapy and Prophylaxis

3.4.1 Chemotherapy

Doxycycline, diethylcarbamazine, ivermectin, and albendazole are available against the microfilariae and the worms, but ivermectin does not show any efficacy against macrofilariae, and albendazole is effective only against the adult worm. The vital symbiosis of Wuchereria, Brugia, and Onchocerca with the bacterium Wolbachia is restricted or inhibited by the use of tetracycline (doxycycline), rifampicine, or azithromycine [58, 10, 8]. Doxycycline significantly reduces the release of VEGF and the dilatation of the lymphatic blood vessels [15] (cf. 1.2 Pathogenesis). The aim of the treatment is to prevent the clinical symptoms of lymphedema, hydrocele and elephantiasis, and, from an epidemiological point of view, to reduce the transmission of the filariae by reducing the production of microfilariae.

3.4.2 Wuchereria bancrofti, Brugia malayi, Brugia timori (lymphatic filariae), and Onchocerca volvulus, Mansonella perstans

Treatment starts with doxcyline (200 mg/day) for 4–6 weeks, then a single dose of diehthycarbazamine (6 mg/kg) plus albendozole (400 mg) is administered, and if there is a simultaneous presence of Onchocercerca and Wuchereria which is true of various regions, ivermectin plus albendozole (400 mg) is used [8, 59]. The treatment regimen is repeated annually for 5 years, and the success of the treatment is monitored by determining the blood and tissue burden of filariae. A reinfection should be prevented through the control and reduction of the spreading of the vector within the first year. For children over 10 years and pregnant women, only treatment with doxycycline, if any, is recommended at present (caution: damage to tooth and bone development by doxycycline).

The efficacy of the doxycycline treatment against *M. perstans* was very high in Mali with a decrease in the microfilaria count to about 23% in the blood after 6 months and to 0% after 12 months [59], whereas a decrease to 62% after 6 months and to 50% after 12 months was measured in the untreated control group. The 12-month efficacy of the doxycycline treatment was 97% (67 of 69 treated individuals), compared with the untreated group with 16% (10 of 63) and with the ivermectin/albendazole group with 19% (5 of 27) [59].

Adverse effects include fever, headache, itching, myalgia, which is high in particular in regions such as Cameroon where *L. loa* is endemic simultaneously. Serious adverse effects with progressive loss of neurological functions and encephalopathy occurred in 1 of 800,000 treated patients [8]. Preliminary treatment with doxycycline prevents the occurrence of severe inflammatory reactions such as acute dermatolymphangioadenitis. Vomiting is a frequent adverse reaction to doxycline treatment [59]. Adverse effects of diethylcarbamazine treatment were more severe the higher the number of microfilariae in the tissue and the more interleukin-6 and lipopolysaccharide-binding protein was released [60].

3.4.3 Loa loa

Diethylcarbamazine is effective only against the adult worm which is killed. Death of the worm is partially associated with a severe Jarisch-Herxheimer reaction which may be accompanied by serious adverse reactions such as encephalopathy which is partly fatal and is caused by microembolisms in the capillaries of the brain [10]. The recommended treatment today is a single administration of ivermectin, 50–200 µg/kg plus albendazole (400 mg), which can be repeated after several months. If the combination of ivermectine and albandazole is used, serious adverse reactions are rare. In patients with a microfilaria burden of < 8,000/ml, the occurrence is very low. Allergic adverse reactions can be suppressed by the administration of cortisone. A simultaneous administration of tetracycline is not required since no Wolbachia are present in *L. loa* [7, 10]. Moxidectine, which shows strong efficacy

Fig. 6. Subcutaneously remaining dead *L. loa* microfilaria – picture of larva migrans. The only clinical symptoms which persist are the induration and hyperpigmentation (photo: Dr. R. Krippner, German Embassy, Yaounde, Cameroon, 2006).

against microfilariae, could be introduced as new medicinal product for the treatment of macrofilariae [61]. Figure 6 shows a dead microfilaria in the skin – a so-called larva migrans – in a Cameroonian woman.

3.4.4 Prophylaxis of Arthropod Stings

Treatment of the Breeding Grounds of the Vectors
DDT (dichlorodiphenyltrichloroethane) is no longer recommended because of the neurological adverse effects in humans and the resistance of some mosquito species. In areas near human settlements it is recommended to cover free water surfaces, including open latrines, with polystyrene beads in order to suffocate the developing mosquito larvae. This measure helped to reduce the stinging rate in a community from around 25,000 per year to around 500 per year [62]. Another very effective measure is the removal of potential breeding grounds of mosquitoes by draining.

Mosquito Bite Prophylaxis
Avoiding mosquito bites is the most effective prophylaxis in many regions in which arboviruses, plasmodiae, and microfilariae are transmitted simultaneously by mosquitoes [13]. Anopheles stings can be prevented in twilight which, in Africa, lasts from 4:00 to 6:30 am and from 4:00 to 6:30 pm. The bites of Culicoides with diurnal and nocturnal activity are hard to prevent, while Culex and Aedes are active during the night.

Prevention of Horse Fly Bites
Horse flies sting in the daytime. They are found in shady areas, waiting for an opportunity to sting. Animals, including monkeys, attract horse flies. Such areas should therefore be avoided. Clothes that are light in color and have stripes reduce the probability to attract horse flies. A horse fly sting is very painful and is therefore noticed.

Prevention of the Bite of the Black Fly

The habitat of the black fly is in gallery forests of the savannah and the surroundings of rapidly flowing streams/rivers in the rain forest. The black fly occurs in swarms, is found in the shade of the trees, and is aggressive in the daytime. The larvae develop at sites of flowing water with a high concentration of oxygen, i.e. which normally show an open flow of water. Humans and animals are frequent hosts so that a human cycle can develop which, however, can be broken by treating the population affected by administration of ivermectin and albendazole. A black fly sting is extremely painful. Black fly larvae can partly be killed by means of the toxin of *Bacillus sphaericus* or *Bacillus thuringiensis*. However, Bacillus toxin-resistant mosquito larvae have developed in West Africa [63].

Vaccination

The vaccination of jirds (rodents similar to gerbils) with a recombinant protein from maltose binding protein (BM5-MBP) and paramyosin of L3 larvae from *B. malayi* led to immunity after 2 administrations so that after infection with 100 L3 larvae, the number of developing adult worms was reduced, and the latter were smaller [64]. The immunization of BALB/c mice and jirds with maltose binding protein linked to tropomyosine from *O. volvulus* resulted after infection with microfilariae in a reduction of the growth of the adult worms of around 45% [65]. In its amino acid composition, tropomyosine from *O. volvulus* showed 91% homology with that of other nematodes; therefore, this antigen was used for the immunization of mice. The immunity against microfilariae depended on the formation of IgE and could be transferred to other mice [66].

A cellular immune response against *L. loa* was induced in mandrills which were immunized with 50 irradiated L3 larvae. The vaccinated animals showed a Th1 response against L3 whereas non-vaccinated animals showed a Th2 response. After infection with 100 live L3 microfilariae, some animals did not show microfilaremia, which was rated as a protective effect [67]. The vaccination of mandrills with 150 irradiated L3 larvae and artificial infection 60 days later initially did not result in any difference between vaccinated and non-vaccinated mandrills. It was only after 245 days that the microfilaremia was considerably less severe in the vaccinated mandrills [68]. Rats and gerbils were immunized 3 times with recombinant *B. malayi* myosine (molecular weight 73 kDa). After artificial infection with 100 larvae 1 week after the last vaccination, the vaccinated animals showed a reduction in the amount of microfilariae in the blood by 76% and a reduction of worm infestation by around 50% compared to the control animals [69].

These studies showed that a certain degree of immunity can be achieved. However, they indicate that a human vaccine will not be available in the near future. Prevention of microfilaria transmission by prophylactic annual administration of diethylcarbamazine or ivermectin and albendazole or mebendazole in endemic regions is a more effective method of protection than vaccination [31].

3.5 Transmissibility

3.5.1 Blood Transfusion

In acid-citrate-dextrose (ACD) blood, L1 microfilariae of *W. bancrofti* survived for 12 days in a state of good mobility at 4–6 °C. After 21 days they were immobile but could be reactivated by increasing the temperature. After 25 days, they were dead [70]. In citrate blood, microfilariae of *W. bancrofti* stored at 4 °C survived for at least 39 days, those of *B. malayi* survived 47 days, and those of *L. loa* 15 days [71]. Around a third of the microfilariae of *L. loa* survived for 21 days in ACD blood [54]. Consequently, microfilariae present in a blood donation are theoretically able to survive up to the time of transfusion.

Transmission of filariosis by transfusion still does not occur when L1 microfilariae are transfused [70], since the further maturation into L3 microfilariae is processed only in the vector but not in humans. *M. perstans* filariae, which were transmitted by transfusion, were rapidly eliminated from the recipient's blood, and no symptoms or eosinophilia occurred after 4 months [72, 73]. On the other hand, Gönnert [55] and also Nutman [74] described that *M. perstans* microfilariae were detectable in the recipient up to 3 years after transfusion. In an analysis of the transmission of microfilariae by blood transfusion in India, the recipients of 11,572 transfusions were followed up for 15 months. 5 of the donors were carriers of filariae, 4 of the recipients were infected, and in 2 of the recipients and the donors, filariae were detectable during the entire observation period. The conclusion was that even if transmission of the microfilariae does not lead to development of the adult worm, blood donors with known filaria infections should be excluded from donating blood [75]. In the USA, the transfusion of blood from a regular donor transmitted *M. ozzardi* after the donor had spent a holiday in the Caribbean [52], as discussed in 1.3 and 2.1.

As for plasma, transmission of microfilariae by fresh frozen plasma (FFP) can be ruled out since microfilariae do not survive the freeze-thaw cycle. In the case of stab injury and occupational infection, so far no transmission of microfilaria by injury during surgical intervention or needle stick has been reported.

3.6 Frequency of Administration of the Blood Products and Amount Administered

FFP is not infectious in the case of filariae since the parasites are killed during the freeze-thaw cycle (cf. 3.5). As for red blood cell concentrates, no statements can be made on the

frequency and amount since figures are not available for Germany. Since L1 larvae can survive in humans for 6 weeks and up to 37 months, the deferral of blood donors for 6 months due to the risk of acquiring malaria after a stay in a tropical region is not sufficient to exclude filaremia, e.g. by Mansonella [76]. In the case of platelet concentrates, transmission of microfilariae has so far not been reported. L1 larvae are likely to survive the manufacturing process but do not lead to development of the worm since further maturation to the L3 larvae is hampered.

4. Blood Products

4.1 Burden in the Starting Material and Testing Methods

In 1987, in endemic areas such as Ibadan, Nigeria, 3.5% of the blood donors tested had *L. loa* microfilaremia while 7.8% were infected with *P. falciparum* [77]. None of 364 blood donors in Maiguguri, Nigeria, had a filaria infection [78]. Out of 1,259 blood donors in Ile-Ife, Nigeria, 2 (0.16%) were carriers of microfilariae [79]. In the blood bank at a maximum care hospital in Bangkok, Thailand, no filarial infections were detected in blood donors from 1999 to 2009 [80]. The prevalence in other endemic areas is described in Section 1.3 'Epidemiology'. The microfilaria burden in the starting material therefore varies considerably in endemic areas but should be generally considered as very low if at all present. If needed, microfilariemia could be detected in blood donations by NAT (cf. 1.4 Genome detection).

4.2 Possibility of Removal and Inactivation of the Infectious Agent

Microfilariae normally circulate in the blood. On day 1 of a blood collection, 80% of *L. loa* microfilariae were retained by filtration via a 40 μm blood filter, while on day 21 this rate was 92% [54].

Microfilariae in the L1 or L3 stadium could be kept alive in medium containing reduced glutathion, human cells and human serum (specific batches are required) [81], and could be used for spike assays. Herbal extracts impairing the viability of microfilariae have been investigated using this method [82]. Spike assays could therefore be performed with a high workload to test removal or inactivation.

The inactivation of microfilariae under the aspect of plasma or plasma derivatives has so far not been described. For non-cellular blood products, a freeze-thaw procedure is sufficient to kill the microfilariae. Only around 6% of the microfilariae are killed during cryopreservation in the presence of 6% dimethyl sulfoxide (DMSO) and 15% serum, as used for the storage of stem cells [83]. Irradiation with gamma rays up to 10.5 Gy does not kill microfilariae of *W. bancrofti* [84].

Treatment with 5 mg/ml diethylcarbamazine in vitro caused disintegration of microfilariae of *W. bacrofti* [85], and treatment with 2–10 μmol/l mefloquine (Lariam®, Hoffmann–La Roche, Basel, Switzerland) used for malaria prophylaxis killed microfilariae of *B. malayi* after 10 h – 3 days [86].

4.3 Practicability and Validation of Procedures for Removal/Inactivation of Infectious Agents

4.3.1 Susceptibility in Animal Experiment

Animals that can be experimentally infected with *L. loa* include the drill monkey (*M. leukophaeus*) and the mandrill (*M. sphinx*). In Gabon, 7 mandrills were infected with different amounts of L3 larvae of *L. loa*. In 1 of the animals infected with 1,000 L3 larvae, no filariae were found, while in another 4 animals filariemia was detected after approximately 150 days. In the remaining 2 animals whose mothers were infected with microfilariae, the filariae were only detected after 200 days. The filariae burden varied, with up to 10,000 microfilariae/ml; however, after 300 days this amount decreased to 10–100 microfilariae/ml in all 6 animals. The monkeys showed no clinical symptoms, no leukocytosis, and no eosinophilia [87]. In conclusion, a sufficiently validated animal model is not available. Antigen tests and PCR (cf. 1.4 Detection methods) are suitable for detecting a reduction in the pathogen burden. However, they are not suitable for ruling out residual infectivity.

5. Assessment

Arbonematodes are widespread in tropical regions with rain forests or gallery forests. They can be present in high numbers in infected individuals. Filariae are very rarely found in Germany, and up to now, no reports exist stating that these parasites were transmitted by blood transfusions in Germany. The larvae of the filariae present in a blood donation, if any, are L1 larvae which, depending on the amount and type, die in the new human host within a few weeks up to several months, and are unable to mature into worms. From this point of view, the transmission of microfilariae by blood transfusion would be a self-limiting event and thus does not require any further action for the protection of the recipient. Whether the prevalence of microfilariae in the mosquito and horse fly vectors will increase in the course of global warming, which would make transmission to humans in Germany more likely, should be monitored. Asian primates and cats are known as non-human hosts for Brugia, and African primates for *L. loa*. From an epidemiological point of view, these animals are not important for a transmission cycle in Germany. Routine testing for microfilariae or an extension of the deferral for blood donors after travelling in endemic regions is not required.

This paper was completed on November 22, 2011, and approved by the German Advisory Committee Blood (Arbeitskreis Blut) on March 30, 2012. It was compiled by the members of the subgroup 'Assessment of Pathogens Transmissible by Blood' of the German Advisory Committee Blood (Arbeitskreis Blut):

Prof. Dr. Lutz Gürtler
Dr. Ursula Bauerfeind
Dr. Johannes Blümel
Prof. Dr. Reinhard Burger
Prof. Dr. Christian Drosten
Dr. Albrecht Gröner

Dr. Margarethe Heiden
Prof. Dr. Martin Hildebrandt
Prof. Dr. Dr. Bernd Jansen
Dr. Thomas Montag-Lessing
Dr. Ruth Offergeld
Prof. Dr. Georg Pauli
Prof. Dr. Rainer Seitz
Dr. Uwe Schlenkrich
Dr. Volkmar Schottstedt
Dr. Johanna Strobel
Dr. Hannelore Willkommen

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