Repeated high doses of avermectins cause prolonged sterilisation, but do not kill, Onchocerca ochengi adult worms in African cattle

Citation for published version:
Bronsvoort, M, Renz, A, Tchakouté, V, Tanya, VN, Ekale, D & Trees, AJ 2005, 'Repeated high doses of avermectins cause prolonged sterilisation, but do not kill, Onchocerca ochengi adult worms in African cattle', Filaria journal, vol. 4, pp. 8. https://doi.org/10.1186/1475-2883-4-8

Digital Object Identifier (DOI):
10.1186/1475-2883-4-8

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Filaria journal

Publisher Rights Statement:
2005 deC Bronsvoort et al; licensee BioMed Central Ltd.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Repeated high doses of avermectins cause prolonged sterilisation, but do not kill, Onchocerca ochengi adult worms in African cattle

Barend M deC Bronsvoort*1,4, Alfons Renz2,5, Virginia Tchakouté1, Vincent N Tanya3,6, David Ekale2 and Alexander J Trees1

Address: 1Veterinary Parasitology, Division of Parasite and Vector Biology, Liverpool School of Tropical Medicine/Faculty of Veterinary Science, University of Liverpool, Pembroke Place Liverpool,UK, 2Fachgebiet Parasitologie, Universität Hohenheim, Germany, 3Institut de Recherches Agricoles pour le Développement, Ngaoundere Cameroon, 4Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, UK, 5Universität Tübingen, Friedhofstrasse 73, 72074 Tübingen, Germany and 6Institute of Agricultural Research for Development, Regional Centre of Bambui, B.P. 51 or 80, Bamenda, Cameroon

Email: Barend M deC Bronsvoort* - mark.bronsvoort@ed.ac.uk; Alfons Renz - Alfons.Renz@uni-tuebingen.de; Virginia Tchakouté - pngtchakoute@lineone.net; Vincent N Tanya - vntanya@yahoo.com; David Ekale - dibongoekale@yahoo.fr; Alexander J Trees - trees@liv.ac.uk
* Corresponding author

Abstract

Background: Ivermectin (Mectizan™, Merck and CO. Inc.) is being widely used in the control of human onchocerciasis (Onchoverca volvulus) because of its potent effect on microfilariae. Human studies have suggested that, at the standard dose of 150 µg/kg an annual treatment schedule of ivermectin reversibly interferes with female worm fertility but is not macrofilaricidal. Because of the importance of determining whether ivermectin could be macrofilaricidal, the efficacy of high and prolonged doses of ivermectin and a related avermectin, doramectin, were investigated in cattle infected with O. ochengi.

Methods: Drugs with potential macrofilaricidal activity, were screened for the treatment of human onchocerciasis, using natural infections of O. ochengi in African cattle. Three groups of 3 cows were either treated at monthly intervals (7 treatments) with ivermectin (Ivomec®, Merck and Co. Inc.) at 500 µg/kg or doramectin (Dectamax®, Pfizer) at 500 µg/kg or not treated as controls. Intradermal nodules were removed at 6 monthly intervals and adult worms were examined for signs of drug activity.

Results: There was no significant decline in nodule diameter, the motility of male and female worms, nor in male and female viability as determined by the ability to reduce tetrazolium, compared with controls, at any time up to 24 months from the start of treatments (mpt). Embryogenesis, however, was abrogated by treatment, which was seen as an accumulation of dead and dying intra-uterine microfilariae (mf) persisting for up to 18 mpt. Skin mf densities in treated animals had fallen to zero by <3 mpt, but by 18 mpt small numbers of mf were found in the skin of some treated animals and a few female worms were starting to produce multi-cellular embryonic stages. Follow-up of the doramectin treated group at 36 mpt showed that mf densities had still only regained a small proportion of their pre-treatment levels.

Conclusion: These results have important implications for onchocerciasis control in the field. They suggest that ivermectin given at repeated high doses may sterilise O. volvulus female worms for prolonged periods but is unlikely to kill them. This supports the view that control programmes may need to continue treatments with ivermectin for a period of decades and highlights the need to urgently identify new macrofilaricidal compounds.
Background
An estimated 17.5 million people in Africa and a further 140,000 in the Americas are infected by the filarial parasite *Onchocerca volvulus*, the cause of human river blindness [1]. The disease and its vector, *Simulium damnosum s.l.*, have been the target of the largest successful and sustained vector-control programme the world has known, the Onchocerciasis Control Programme in West Africa (OCP) [2]. However in 1987 the veterinary endectocide, ivermectin, was registered as a safe and effective microfilaricidal for human use against *O. volvulus* as an oral preparation, Mectizan™. The manufacturers, Merck and Co. Inc., via their Mectizan™ Donation Programme are committed to provide ivermectin free of charge to the port of entry of endemic countries ‘for as long as is necessary’ to aid the control of onchocerciasis. The African Programme for Onchocerciasis Control (APOC) [3] utilises ivermectin as its main tool to eliminate onchocerciasis as a public health problem. It aims to distribute ivermectin once a year through a sustainable, community-directed distribution system (CDTS).

The macrocyclic lactones are effective against a wide range of ecto- and endoparasites of animals and man. They are grouped into two major chemical families: the avermectins (including ivermectin and doramectin) and the milbemycins (including moxidectin). Ivermectin is derived from the actinomycete, *Streptomyces avermitilis*, and was originally developed as a veterinary endectocide against endo- and ectoparasites. Doramectin is a fermentation product from a mutant *S. avermitilis* strain. Its spectrum of activity is nearly the same as ivermectin [4] but its lipophilic cyclohexyl moiety at the 25-position produces a longer tissue half-life of 89 hours [5].

The efficacy of ivermectin against the skin dwelling larval stage of *O. volvulus* which causes blindness and skin lesions is well established [6-8]. However, its action against adult male and female worms is the subject of debate. Human studies with *O. volvulus* using a single dose at 150 µg/kg [9] produced no increased mortality of the adult worms and reversible affects on their fertility. Repeated 6-monthly [10-13] or 3-monthly treatments [14] produced conflicting results, but a proportion of female worms recovered their fertility 12–18 months after the last treatment and mf loads were 6–7% of pre-treatment loads 12 months after the last treatment, rising to between 9–32% at 18 months [15]. Epidemiological analysis and mathematical simulations of annual ivermectin treatments in man suggest that there is approximately a 30% reduction in mf production [16]. These authors, however, were not able to differentiate between the possible biological mechanisms such as a general loss of fecundity, or death of a proportion of the adult worms, or ivermectin facilitated immunity.

In this paper, we describe a controlled therapeutic trial in which the effects of high, repeated doses of ivermectin or doramectin were investigated against the African cattle parasite *O. ochengi* for up to 36 months after the first treatment. This species is phylogenetically closely related to *O. volvulus* for which it provides an excellent analogue [17] and was formerly adopted by the World Health Organisation (WHO) tertiary drug screening system for macrofilaricides until 2000. Previous studies have indicated both its practical merits and its predictive value [18-20].

Materials and methods
Between February and March 1994, nine 4–6 year old Gudali cows (*Bos indicus*), each with more than 20 palpable *O. ochengi* nodules in the ventral abdominal skin, were purchased from local markets in the Adamoua province of Cameroon. They were kept at pasture on the research station of the Institut de Recherches Agricole pour le Développement (IRAD) (formerly the Institut de Recherches Zootechniques et Vétérinaires), Wakwa, 10 km S.W. of Ngaoundere, at an altitude of 1000 m where the annual transmission potential (ATP, measured in L3s/person/year) for *O. ochengi* is low, around 600 L3s (Renz and Bronsvoort unpublished observations). Treatments began in October 1994, and nodecortomies and 3-monthly skin biopsies were continued for 24 mpt. In July of 1997 (36 mpt) the experiment was terminated.

Treatment regime
The cows were cast in lateral recumbency and the nodule distribution on each side of the ventral abdomen was mapped on a standardised recording sheet to allow tracking of individual nodules. The cows (mean weight = 379 kg) were ranked in order of their number of nodules and assigned to one of 3 groups, giving an even distribution of nodules between groups (Table 2). The cows were treated with either a subcutaneous inject of 500 µg/kg ivermectin, or a subcutaneous inject of 500 µg/kg doramectin or not treated to act as controls. Treatments were repeated at monthly intervals for 7 treatments.

Sampling regime
At 3-monthly intervals 3 superficial skin biopsies (between 10–20 mg) per animal were taken from a shaved patch of skin on the ventral mid-line between the umbilicus and the udder. They were incubated in Roswell Park Institute Medium (RPMI) for 24 hours followed by collagenase at 37°C for 24 hours, using the protocol of Renz et al. [18]. The majority of mf escaped from the skin in the first 24 hours of incubation with the remaining mf found after digestion of the skin with collagenase. The geometric mean number of mf per 100 mg of skin from the three biopsies for each animal using the log+1 transformation. The mean per group was calculated in a similar way. All animals were sampled on the same morning.
O. ochengi nodules numbers were assessed by careful palpation of the skin of each cow. The distribution of nodules was recorded prior to treatment and about 20 nodules per cow were permanently marked with three dots around the nodule using green tattooing ink. This ensured identification of sites where nodules had been should they disappear completely following treatment. Nodule numbers were re-counted at 6 monthly intervals until 24 mpt (8/9 animals). After recording nodule distribution, four nodules per animal were removed under local anaesthetic and the wounds closed with a catgut suture and treated with Negasunt® (Bayer plc) powder to control fly strike. Two nodules per cow were preserved in 10% formal saline for histological examination. The remaining 2 nodules per cow were examined the same day by the triple assay described by Renz et al. [18], with the following slight modifications.

**Triple assay of live female and male worms**

In brief, the nodules were trimmed of any host tissue and measured along their longest axis to give the ‘nodule diameter’. Each nodule was then cut open on a hollow slide in phosphate buffered saline and the worm contents carefully expressed. The male worms were teased free and placed individually in the wells of a 24 well plate with RPMI. The anterior 5–10 mm of each female worm was amputated and placed in a separate well and classed as either 1, young (transparent white, little incrustations on the cuticle); 2, mature (yellow-brownish, with incrustations); 3, old (dark brownish, strong incrustations); or 4, calcified (black and calcified).

After incubation (30 minutes at 37°C), the motility of male worms and anterior ends of female worms were scored after 30 seconds of observation on a 3 point scale (0 = no movement; 1 = weak, slow or intermittent movement; 2 = vigorous movement). Individual worm viability was then assessed by the MTT/formazan assay [18,21]. The optical density of the formazan reduction product was determined at 492 nm (OD_{492}, 'Cambridge Life Instruments photo-spectrometer'). Mean OD_{492} readings for the total male worm or anterior ends of female worms were used until 18 mpt after which the mean OD_{492} / 10 mm of worm was calculated.

The remaining (posterior) part of each female worm was then washed into a mortar with 1.8 ml of PBS (final volume 2 ml), gently crushed with a pestle and a sample transferred to a Fuchs-Rosenthal chamber (3.2 µl) to determine an embryogram following the method of Schulz-Key [22]. A count was made of the normal and abnormal/pathologic embryonic stages and intra-uterine mf. Oocytes were also counted, but the repeatability of their counts was not reliable and they were not included in the final analysis.

**Statistical Analyses**

Statistical analyses were performed using 'Minitab' and BMDP computer packages. The nodule counts, nodule diameters and the MTT reduction/formazan formation results were analysed using a two-way repeat measures ANOVA between groups and timepoints. Worm age, motility scores and the percentage pathological stages at each timepoint were compared using a Kruskal-Wallis non-parametric one-way ANOVA. All statistical tests were interpreted at the 5% level of significance.

**Results**

Of the original nine cows at the start of the study, one control animal, no. 17, had to be slaughtered after the 12 mpt sampling on welfare grounds. Two more animals, one control and one ivermectin treated animal, were removed for different reasons after the 24 mpt sampling and regular observations were terminated at this point. However,
where possible skin mf loads were monitored and nodules collected from the remaining six animals until 51 mpt.

**Microfilariae**
Following treatment, the mf density in the skin declined sharply and no skin mf were found at 3 mpt (Table 1). The control animals maintained substantial mf loads throughout the study, although their loads fluctuated considerably. Small numbers of mf began reappearing in 4/6 of the treated animals between 15 and 21 mpt.

**Nodule counts, diameter and female age score**
Table 2 gives the mean nodule counts, the mean nodule diameter and the median female age score (male age scores were not included) for the 3 groups before and 24 months after the start of treatment. There was no significant difference in nodule counts or nodule diameters between the groups or time points.

**Motility, number of males and females per nodule and viability as measured by formazan formation**
Male *Onchocerca* worms are mobile and although the number per female varied during the experiment, the ratio of males to females remained around one (with one female worm to each nodule). The male and female median motility scores are summarised in Table 3 and Figure 1. The median motility of male worms remained at 2 throughout the study except at 18 mpt for the ivermectin group. Similarly, for female anterior ends, their motility was 2 except at 24 mpt when this dropped. A Kruskal-Wallis ANOVA comparing the group medians for this time-point was not significant ($p = 0.20$).

From Figures 1a and 1b there is clearly no drug effect on worm viability/OD$_{492}$ of either males or females. There is an effect of time with mean OD$_{492}$ readings dropping after 18 mpt. This effect is associated with the change to a standardisation of the viability/OD$_{492}$ per 10 mm of worm length.

**Embryograms**
The results of the embryograms are given in Figure 2. Normal embryonic stages (small morulae, large morulae, horseshoes, pretzels or coiled microfilariae) disappeared from the two treated groups by 6 mpt and failed to reappear in the female worms. Intrauterine mf disintegrated steadily, first becoming vacuolated and non-motile, and eventually becoming ghost forms. This sterilizing effect persisted in 4 of the 6 treated animals for up to 24 months from the start of treatment. In two of the doramectin treated animals (i.e. 2/6 nodules), classed as old, was recovered at both the 18 and 24 mpt examinations, with large numbers of normal embryonic and intra-uterine mf amongst the pathological stages still in the uteri. In the ivermectin-treated animals, no fertile nodules were found, but the small number of mf in the skin in one animal (no. 14) indicates the presence of at least one fertile worm couple.

**Discussion**
Previous drug trials [18,23] have demonstrated the advantages of the *O. ochengi* screen in cattle in which individual

---

Table 1: Geometric mean (3 skin snips) *O. ochengi* mf density+1 per 100 mg skin in cattle following 7 monthly treatments with ivermectin 500 µg/kg subcutaneously, doramectin 500 µg/kg subcutaneously or untreated controls. Animals lost to follow-up are indicated in the table.

| ID  | 0 mpt  | 3 mpt  | 6 mpt  | 9 mpt  | 12 mpt | 15 mpt | 18 mpt | 21 mpt | 24 mpt | 36 mpt |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Control |        |        |        |        |        |        |        |        |        |        |
| 17   | 3803   | 635    | 203    | 0      | 18     | a      |        |        |        |        |
| 19   | 841    | 260    | 1515   | 774    | 1135   | 604    | 1698   | 185    | 257    | c      |
| 22   | 4578   | 1583   | 911    | 712    | 767    | 291    | 982    | 975    | 1462   | 281    |
| Ivermectin | 14     | 509    | 0      | 0      | 0      | 0      | 0      | 8      | 2      |        |
| 18   | 671    | 0      | 0      | 0      | 0      | 2      | 0      | 0      | 0      | b      |
| 20   | 240    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |        |
| Doramectin | 12     | 6901   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 5      |
| 15   | 1607   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 16     |
| 23   | 146    | 0      | 0      | 0      | 0      | 3      | 2      | 0      | 0      | 75     |

a: Slaughtered following tetracycline therapy for dermatophilus infection, b: slaughtered due to septic arthritis, c lost to follow-up
animals are sequentially sampled and worm viability is determined.

As in previous studies in cattle [18], the skin mf density declined to zero following treatment with ivermectin and the same result was found with doramectin. Although mf began reappearing around 18 mpt only one animal, no. 12, showed a consistent increase in mf density and therefore the presence of at least one fertile worm couple. An interesting observation was the disappearance of mf from the skin of a control animal, no. 17. This was associated with repeated treatments of oxytetracyclines for a severe mycotic dermatitis (Dermatophilus congolensis) skin infection. Treatment failed to control the skin infection and the animal was culled on welfare grounds after the 12-mpt sampling. The macrofilaricidal effect of oxytetracyclines was subsequently demonstrated [23].

In spite of being given at 2.5 times the recommended dose for cattle and at monthly intervals for 7 treatments, neither ivermectin nor doramectin reduced nodule diameter, male or female worm number or male motility compared with controls. Female motility appeared to be compromised in the doramectin treated group by 24 mpt, though this was not statistically significant. The viability/OD492 of female (a) and male (b) O. ochengi formazan formation after MTT reduction before and up to 24 months after treatment (from 18 months after the start of treatment OD492 is standardised for 10 mm of length). □ = control, □ = ivermectin, ■ = doramectin. (N.B. The mean OD492 reading for males was first calculated for each nodule and this was then used to calculate the group means given in Figure 1b) Foot notes. (a) Two-way ANOVA for effect of timepoint and treatment group: Interaction effect between group and timepoint p = 0.44; group effect p = 0.62; effect of timepoint p = 0.02. (b) Two-way ANOVA for effect of timepoint and treatment group: Interaction effect between group and timepoint p = 0.44; group effect p = 0.62; effect of timepoint p = 0.02.

The main effect of both ivermectin and doramectin on the adult female worm was on the development of the multicellular embryonic stages and the intra-uterine mf, as in man [10,12,13] where repeated treatments of ivermectin at 150 µg/kg every 6–12 months were reversible after 9–18 months though fecundity had been reduced. Interestingly, the number of intra-uterine mf rose sharply in both treated groups as dead and dying mf accumulated in the uteri of the worms. Over the same period the embryonic stages disappeared from the uteri. Certainly some of these
must have continued to develop through to mf in order to explain these changes [26]. Why the degradation and disappearance of dead mf from the uteri is so protracted, is unclear, but it may be important in preventing fertilisation of the female [11,27], as this is the stimulation for a new cycle of oogenesis [28]. Alternatively, the uteri may be paralysed as happens to the pharyneal muscles of *Haemonchus contortus* [29].

Observations in man noted a disappearance of *O. volvulus* males from nodules following multiple treatments with ivermectin [14,30]. In addition, after treatment with other macrofilaricidal drugs, fewer males were recovered from *O. ochengi* nodules [18] and this is considered a useful, early indicator of macrofilaricidal activity. Our study, however, is in agreement with observations on *O. volvulus* in man [11,12] as we found male numbers to vary over time with no clear decline. Further, the male worms maintained their motility and viability (OD492) throughout the 24 months of the study. The effects of avermectins on male fertility were not examined here.

Our study has demonstrated that with higher doses of ivermectin or doramectin, repeated at monthly intervals, embryogenesis can be disrupted and possibly permanently impaired. Even 18 months after the last treatment none of the removed ivermectin treated nodules showed signs of recommencing embryogenesis. At 24 mpt, 2/6 nodules in the doramectin group did appear to regain some reproductive capability.

In previous studies of age-specific nodule counts [31] it has been suggested that nodules continue to be acquired throughout the life of the cow while their mf loads level off around 4–6 years of age. From the prophylactic studies in cattle [19,32] it is clear that ivermectin and moxidectin can prevent the development of L3 larvae in the host for at least 3 months after a single treatment. The corals at Wakwa have a known low ATP of around 600 L3’s per animal per year as opposed to 24,000 L3’s per year near the *Simulium* breeding sites (Renz and Bronsvoort unpublished observations). In our study although there was a low level of transmission in the area, the nodule burden of the animals did not alter significantly up until 24 mpt after allowing for the removal of 20 nodules per animal. An alternative hypothesis could be that there is some form of density-dependent regulation of the adult worms with only very low turnover of the adult population. This theory is supported by the observation that control animals in the current experiment failed to acquire new nodules in the first year as determined by the regular 6 monthly counts. In addition different trials where L3’s were experimentally inoculated into calves have failed to produce a clear positive correlation between the number of infective larvae inoculated and the number of nodules that subsequently developed [33,34]. If in fact there is some form of density dependent regulation of the adult population with individual worm burdens determined by some host-parasite balance then it may actually be more beneficial to sterilise the female worm, thus preventing the majority of new infections from developing.

The sterilising effect of high doses of ivermectin, combined with their prophylactic potential [19], will ensure the usefulness of this drug and/or related compounds. With community-directed distributions being set up under the APOC scheme there will be a much improved potential for timing treatments and/or increasing the frequency of treatments to maximise these other useful effects. High doses and increased frequency of treatment has been shown here to reduce female worm fertility in *O.*
ochengi and similar results have been demonstrated in humans for *O. volvulus* [35]. This potentially could help to reduce the length of control programmes though it is doubtful whether high coverage levels could be sustained long enough to achieve worldwide eradication [36]. In addition, work on *O. ochengi* suggests that people receiving ivermectin are unlikely to develop immunity to *O. volvulus* and are therefore highly susceptible should drug distribution cease [32]. However, WHO itself recognises that ivermectin by itself can not completely interrupt transmission of *O. volvulus* [37-39] and therefore work should continue to find and develop an effective macrofilaricidal that would be used in combination with ivermectin.

In conclusion, although this experiment has failed to demonstrate that high and repeated doses of ivermectin or doramectin are macrofilaricidal, it has shown that they both have a profound effect on embryogenesis producing a prolonged period of sterility in female worms. However, simulation modelling suggests that ivermectin alone will not interrupt transmission [36]. This appears to be born out by epidemiological studies from the field where the conclusion was that repeated ivermectin mass annual treatment will not lead to the elimination of transmission of onchocerciasis from West Africa though data on 6-monthly treatments was not sufficient to draw a conclusion [39]. There is, therefore, still an urgent need to continue the search for a safe effective macrofilaricidal compound.

**Authors’ contributions**

**BB:** Carried out the field work and project management in Cameroon and was responsible for data analysis and preparation of the manuscript

**AR:** Responsible for project inception and design and preparation of the manuscript

**VNT:** Responsible for field work, project set-up and design and overall project coordination in Cameroon

**VT:** Responsible for field work and project set-up

**DE:** Responsible for analysis and interpretation of field samples in the laboratory

**AT:** Responsible for project inception and design and preparation of the manuscript

**Note**

1. Minitab Inc, http://www.minitab.com

2. BMDP Statistical Software, Inc., Cork Technology Park, Cork, Ireland.

**Acknowledgements**

This investigation was funded by the MACROFIL programme of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (ID no. 910583). Histological sections were prepared by Mrs. H. Böggering, University of Tubingen. Pfister (Germany) are thanked for donating doramectin. We thank Dr. Banser, director of IRAD, for his support. We acknowledge Jean Ebene for his technical support. We are most grateful to Abbo Bakar, Abbo Soudi and Yakouboi for their livestock skills, dedication and great enthusiasm. The authors would also like to thank Dr. Brian Duke for his helpful comments.

**References**

1. WHO: Onchocerciasis and its control. In *WHO Technical Report Series Volume 852*. Geneva, WHO; 1995.

2. Molyneux DH, Davies JB: *Onchocerciasis control: Moving towards the millennium*. *Parasitol Today* 1997, 13:418-425.

3. Remme JHF: *The African Program for Onchocerciasis Control - Preparing to Launch*. *Parasitol Today* 1995, 11:403-406.

4. Shoop NL, Mrozik H, Fisher HM: *Structure and activity of avermectins and milbemycins in animal health*. *Veterinary Parasitology* 1995, 59:139-156.

5. Goudie AC, Evans NA, Gratton FAF, Bishop BF, Gibson SP, Holdom KS, Kaye B, Wicks SR, Lewis P, Weatherley AJ, Bruce CI, Herbert A, Seymour DJ: *Doramectin - a potent novel endectocide*. *Veterinary Parasitology* 1993, 49:5-15.

6. Awadzi K, Opoku NO, Addy ET, Quarry BT: *The Chemotherapy of Onchocerciasis*. 19. *The Clinical and Laboratory Toler-ance of High-Dose Ivermectin*. *Trop Med Parasitol* 1995, 46:131-137.

7. Richards FO, Flores RZ, Duke BOL: *Dynamics of Microfilariae of Onchocerca-Volvulus over the 1st 72 Hours after Treatment with Ivermectin*. *Trop Med Parasitol* 1989, 40:299-303.

8. Greene BM: *Modern Medicine Versus an Ancient Scourge - Progress toward Control of Onchocerciasis*. *J Infect Dis* 1992, 166:1-21.

9. Duke BOL, Zeaflorres G, Munoz B: *The Embryogenesis of Onchocerca-Volvulus over the 1st Year after a Single Dose of Ivermectin*. *Trop Med Parasitol* 1991, 42:175-180.

10. Duke BOL, Zeaflorres G, Castro J, Cugn EV, Munoz B: *Comparison of the Effects of a Single Dose and of 4 6-Monthly Doses of Ivermectin on Adult Onchocerca-Volvulus*. *Am J Trop Med Hyg* 1991, 45:132-137.

11. Chavasse DC, Post RJ, Lemoh PA, Whitworth JAG: *The Effect of Repeated Doses of Ivermectin on Adult Female Onchocerca-Volvulus in Sierra-Leone*. *Trop Med Parasitol* 1992, 43:256-262.

12. Klager S, Whitworth JAG, Post RJ, Chavasse DC, Downham MD: *How Long Do the Effects of Ivermectin on Adult Onchocerca-Volvulus Persist*. *Trop Med Parasitol* 1993, 44:305-310.

13. Klager SL, Whitworth JAG, Downham MD: *Viability and fertility of adult Onchocerca volvulus after 6 years of treatment with ivermectin*. *Trop Med Int Health* 1996, 1:581-589.

14. Duke BOL, Zeaflorres G, Castro J, Cugn EV, Munoz B: *Effects of 3-Month Doses of Ivermectin on Adult Onchocerca-Volvulus*. *Am J Trop Med Hyg* 1992, 46:189-194.

15. Whitworth JAG, Maude GH, Downham MD: *Clinical and paracli-nological responses after up to 6.5 years of ivermectin treatment for onchocerciasis*. *Trop Med Int Health* 1996, 1:786-793.

16. Plassier AP, Alley ES, Boatin BA, Vanoomtassemen GJ, Remme H, Devlas SJ, Bonneux L, Habbema JDF: *Irreversible Effects of Ivermectin on Adult Parasites in Onchocerciasis Patients in the Onchocerciasis Control Program in West-Africa*. *J Infect Dis* 1995, 172:204-210.

17. Trees AJ: *Onchocerca-Ochengi - Mimic, Model or Modulator of Onchocerca-Volvulus*. *Parasitol Today* 1992, 8:337-339.

18. Renz A, Trees AJ, Aulucki D, Edwards G, Wahl G: *Evaluation of Suramin, Ivermectin and Cgp-20376 in a New Macrofilaria-lidal Drug Screen, Onchocerca-Ochengi* in African Cattle. *Trop Med Parasitol* 1995, 46:31-37.
spective study ivermectin prevents calves becoming infected with O- ochengi. Parasitology 1999, 118:195-199.
20. Trees AJ, Wood VL, Bronsvoort M, Renz A, Tanya VN: Animal models - Onchocerca ochengi and the development of chemotherapeutic and chemoprophylactic agents for onchocerciasis. Ann Trop Med Parasitol 1998, 92:S175-S179.
21. Comley JCW, Rees MJ, Turner CH, Jenkins DC: Colorimetric quantification of filarial viability. International Journal of Parasitology 1989, 19:77-83.
22. Schulz-Key H: The collagenase technique: how to isolate and examine adult Onchocerca volvulus for the evaluation of drug effects. Tropical Medical Parasitology 1988, 39:423-440.
23. Angworth NIG, Renz A, Mackensdett U, Henkle-Duhrsens K, Bronsvoort MBD, Tanya VN, Donnelly MJ, Trees AJ: Macrofilaricidal activity of tetracycline against the filarial nematode Onchocerca ochengi: elimination of Wolbachia precedes worm death and suggests a dependent relationship. Proc R Soc Lond Ser B Biol Sci 2000, 267:1063-1069.
24. Cross HF, Bronsvoort BM, Wahl G, Renz A, Achukwi D, Trees AJ: The entry of ivermectin and suramin into Onchocerca ochengi nodules. Ann Trop Med Parasitol 1997, 91:393-401.
25. Cross HF, Renz A, Trees AJ: In-vitro uptake of ivermectin by adult male Onchocerca ochengi. Ann Trop Med Parasitol 1998, 92:711-720.
26. Schulz-Key H, Klager S, Awadzi K, Diallo S, Greene BM, Lariviere M, Aziz MA: Treatment of human onchocerciasis: the efficacy of ivermectin on the parasite. Tropical Medical Parasitology 1985, 36:20.
27. Chavasse DC, Post RJ, Davies JB, Whitworth JAG: Absence of Sperm from the Seminal Receptacle of Female Onchocerca-Volvulus Following Multiple Doses of Ivermectin. Trop Med Parasitol 1993, 44:155-158.
28. Schulz-Key H, Karam M: Periodic reproduction of Onchocerca volvulus. Parasitology Today 1986, 2:284-286.
29. Geary TG, Sims SM, Thomas EM, Vanover L, Davis JP, Winterrowd CA, Klein RO, Ho NFH, Thompson DP: Haemonchus contortus: Ivermectin-induced paralysis of the pharynx. Experimental Parasitology 1993, 77:88-96.
30. Duke BOL, Zeaflora G, Castro J, Cupp EW, Munoz B: Effects of Multiple Monthly Doses of Ivermectin on Adult Onchocerca-Volvulus. Am J Trop Med Hyg 1990, 43:657-664.
31. Trees AJ, Wahl G, Klager S, Renz A: Age-Related Differences in Parasitosis May Indicate Acquired- Immunity against Microfilariae in Cattle Naturally Infected with Onchocerca-Ochengi. Parasitology 1992, 104:247-252.
32. Njonjomet LA, Nfon CK, Gilbert J, Makepeace BL, Tanya VN, Trees AJ: Cattle protected from onchocerciasis by ivermectin are highly susceptible to infection after drug withdrawal. Int J Parasitol 2004, 34:1069-1074.
33. Achukwi, Daiber WH, Renz A, Wahl G, Wanjir S: Prepatency Period and Some Aspects of the Epizootiology of Onchocerca-Ochengi Infestation in Cattle in the Adamawa Plateau, Cameroon. Parasite-J Soc Fr Parasitol 1994, 1:1-10.
34. Trees AJ, Graham SP, Renz A, Bianco AE, Tanya V: Onchocerca ochengi infections in cattle as a model for human onchocerciasis: recent developments. Parasitology 2000, 120:S133-S142.
35. Gordon J, Boussinesq M, Kamengo J, Gardon-Wandel N, Demanga N, Duke BOL: Effects of standard and high doses of ivermectin on adult worms of Onchocerca volvulus: a randomised controlled trial. Lancet 2002, 360:203-210.
36. Winnen M, Plassier AP, Alley ES, Nagelkerke NJD, van Oortmarssen G, Boatin BA, Habbema JDF: Can ivermectin mass treatments eliminate onchocerciasis in Africa? Bull World Health Organ 2002, 80:384-390.
37. Samba EM: Public Health in Action I: the onchocerciasis Control Programme in West Africa. Geneva, WHO, 1994.
38. Seidenfaden R, Fischer A, Bonow I, Ekale D, Tanya V, Renz A: Combined benefits of annual mass treatment with ivermectin and cattle zooprophylaxis on the severity of human onchocerciasis in northern Cameroon. Trop Med Int Health 2001, 6:715-725.
39. Borsboom G, Boatin B, Nagelkerke N, Aghoua H, Akpoboua K, Alley EW, Bissan Y, Renz A, Yameogo L, Remme J, Habbema JD: Impact of ivermectin on onchocerciasis transmission: assessing the empirical evidence that repeated ivermectin mass treatments may lead to elimination/eradication in West-Africa. Filaria Journal 2003, 2:8.