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THERAPEUTIC IMPLICATIONS OF *WOLBACHIA* IN HEARTWORM DISEASE: DO ANTIBIOTICS HELP?

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INTRODUCTION

Heartworm disease in dogs is characterized by chronic inflammatory lesions in the lungs and other organs due to the presence of adults and circulating microfilariae. Furthermore, mild-to-severe side effects are an inevitable consequence of successful adulticide therapy. Following the discovery of the endosymbiont bacteria *Wolbachia* in pathogenic filarial worms, it's biological role in the reproduction and survival of the nematode host has been the subject to intense research (Bandi et al, 2001) . Also, as a gram-negative bacteria, the possible consequences of it's massive release in the filarial-infected host has been evaluated. *Wolbachia* are released both by living worms and following worm death through natural attrition, microfilarial turnover and pharmacological intervention (Taylor et al, 2001). In human and murine models of infection, the release of bacteria has been shown to be associated with the up-regulation of pro-inflammatory cytokines, neutrophil recruitment and an increase in specific immunoglobulins (see The role of *Wolbachia* in this Proceedings). This manuscript will illustrate what is currently known about the effects of antibiotic treatment on filarial worms and on the filarial worm-infected host, including dogs with natural heartworm disease.

WHAT ARE THE EFFECTS OF ANTIBIOTIC TREATMENT ON FILARIAL WORMS?

Wolbachia can be eliminated from filarial worms through antibiotic therapy of the infected host. Numerous studies have shown that various treatment protocols/dosages (tetracycline and synthetic derivatives appear to be the most effective), are able to drastically reduce if not completely remove the endosymbiont from the worm host. Such depletion of *Wolbachia* is then followed by clear anti-filarial effects, including 1) inhibition of larval development: it has been shown that antibiotic treatment of filarial-infected hosts can inhibit molting, an essential process in the maturation of worms from larvae to adult; 2) female worm sterility: antibiotic treatment leads first to a reduction and then to the complete and sustained absence of microfilariae. The detrimental effects on embryogenesis are accompanied by morphological changes within the ovaries and uterus that are often empty or contain degenerating embryos (Figure 1). As stated previously, Bandi et al (1999) reported that *D. immitis* adults taken from naturally-infected dogs that had been treated with 20mg kg^{-1} day^{-1} of doxycycline for 30 days showed morphological alterations of uterine content with a dramatic decrease in the number of pretzels and stretched microfilariae, indicating that bacteriostatic antibiotic treatment was able to block embryogenesis. Initial trials using a six-week course of doxycycline treatment against *O. volvulus* were effective at depleting the bacteria and also resulted in a block of embryogenesis, which persisted for up to two years after the start of treatment. The apparent permanent block in embryogenesis was reflected in sustained reductions in skin microfilariae, the cause of onchocercal disease (Hoerauf et al. 2001, 2003a). Depletion of *Wolbachia* by doxycycline has also been demonstrated in human lymphatic filariasis: in patients infected with *W. bancrofti*, doxycycline administered for 6 weeks at 200 mg/day resulted in a reduction of >95% of *Wolbachia* levels compared to pre-treatment levels. This treatment led to a chronic decline in microfilarial loads, followed by an amicrofilaremia, which was highly significant at 12 months (Hoerauf et al., 2003b). This data suggests that the mode of action of doxycycline is equivalent to that observed in animal models and human onchocerciasis, namely a block in embryogenesis in the adult female worms when *Wolbachia* are absent or at least below a certain threshold. The implications for breaking the life cycle of these parasites are obvious; 3) adulticide effects: this a particularly intriguing aspect of antibiotic treatment of the filarial worm-infected host and one that merits strict attention. Langworthy et al (2000) first reported potential macrofilaricide effects of doxycycline in cattle infected with *Onchocerca ochengi* . The authors show that intermittent therapy with oxitetracycline for a six month period caused the death of adult worms by 9 months post-treatment. More recently, clinical trials in human filariasis have reported extremely promising results: a recent placebo-controlled trial in humans infected with *W. bancrofti* has demonstrated a clear macrofilaricidal effect of doxycycline (Taylor et al., 2005). When administered for 8 weeks at 200 mg/day, the treatment resulted in a complete amicrofilaremia in 28/32 patients assessed and a lack of scrotal worm nests (where adult worms reside) at 14 months post-treatment, as determined by ultrasonography in 21/27 patients. In the other patients, the number of worm nests declined. This was significantly different from placebo patients where lack of worm nests was only observed in 3/27. This is the first report of adulticide activity in a human filarial worm with antibiotics. Could antibiotic treatment have the same effect on *Dirofilaria immitis*?

WHAT ARE THE EFFECTS OF ANTIBIOTIC TREATMENT ON THE FILARIAL WORM-INFECTED HOST?

It is very likely that antibiotic treatment will have some beneficial effects on subjects with filariasis. First of all, those effects described above on the worm will themselves lead to improved clinical presentation: for example, a reduction in migrating microfilariae could lead to a reduction in the ocular inflammation that causes river blindness in *O. volvulus* infection. Even though lymphatic filariasis is due to the activity of the adult worms, there are clinical complications arising

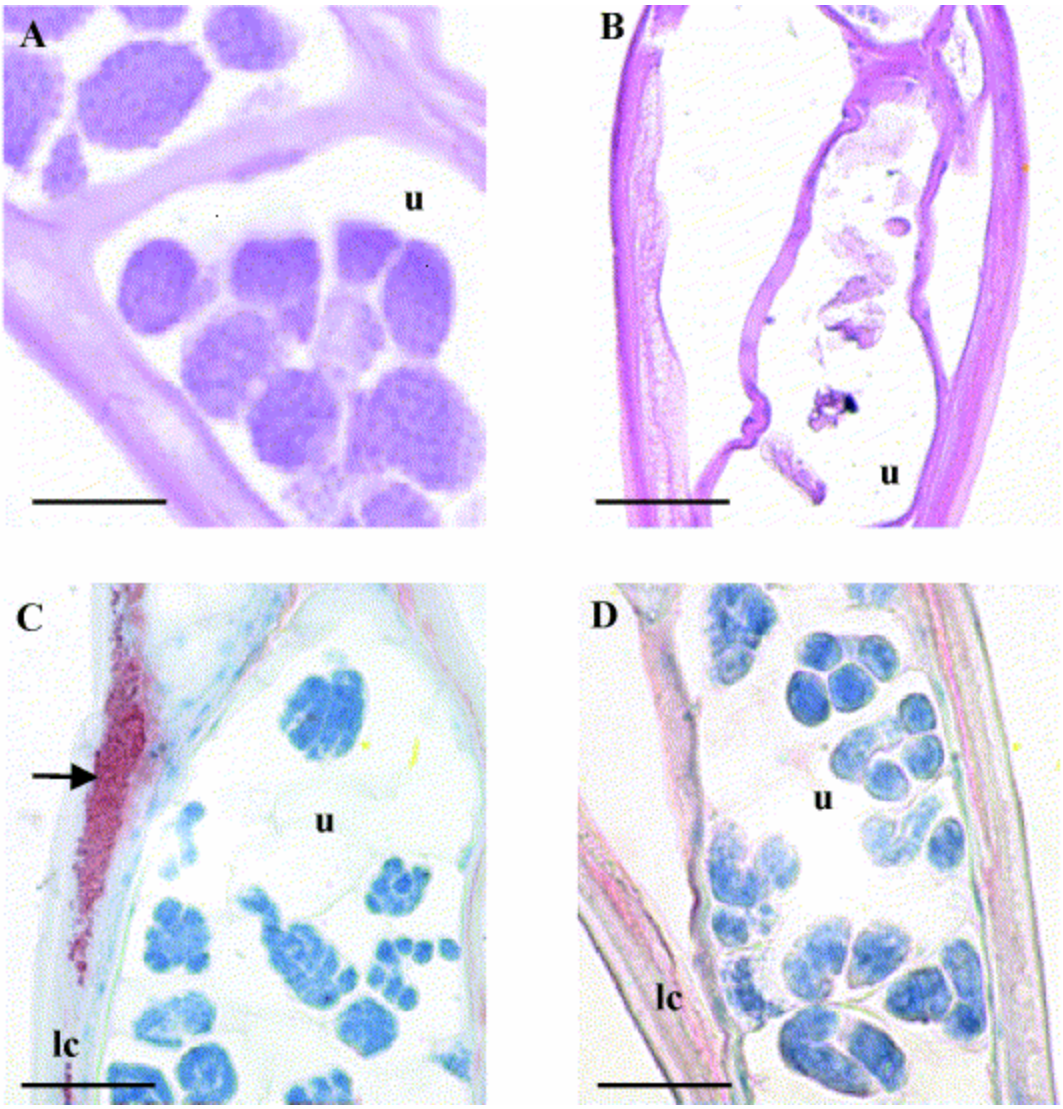

Figure 1. Histology and anti-W SP immunohistochemical staining of *Brugia malayi* females from control jirds (A,C) and jirds treated with tetracycline (B,D). (A) Histology showing normal developing embryos in the uterus (u) of a control female; (B) Histology showing degenerating embryos, replaced with an amorphous material, in the uterus (u) of a treated female; (C) Immunohistochemical staining of *Wolbachia* from a control female showing a cluster of wolbachiae (arrow) in the lateral cord (lc); (D) Immunohistochemistry of a treated female showing a lack of staining for *Wolbachia* in the lateral cords (lc) (with permission from Casiraghi et al, 2003).

from long-term amicrofilariemia is desirable and, with doxycycline, attainable.

However, if we consider *Wolbachia* as a potential cause of inflammation in the course of filarial disease, depletion of the bacteria may be beneficial, independently of its effect on the worm. Periodic treatment with microfilaricidal drugs like ivermectin is currently used to control many endemic areas for human filariosis. There are reports of mild-to-severe side effects of such treatment that have been correlated with the massive release of *Wolbachia* following mf death. Preventive treatment with doxycycline to reduce *Wolbachia* load in microfilariae would undoubtedly decrease/prevent such adverse effects.

There is little data concerning the effects of antibiotic treatment in dogs with natural heartworm disease. We know however, from Bandi et al (1999), that such treatment drastically reduces *Wolbachia* loads in *D. immitis.* We therefore decided to study the effect of doxycycline treatment before adulticide therapy with melarsomine in 5 dogs with natural HW infection. A further five infected dogs were used as negative controls. Following owners' consent, treated dogs received doxicycline at 10 mg kg^{-1} for 30 days before adulticide therapy, while the other group did not receive any medication. Adulticide therapy was carried out according to the American Heartworm Society (AHS) guidelines. Cage rest and heparin were applied to all

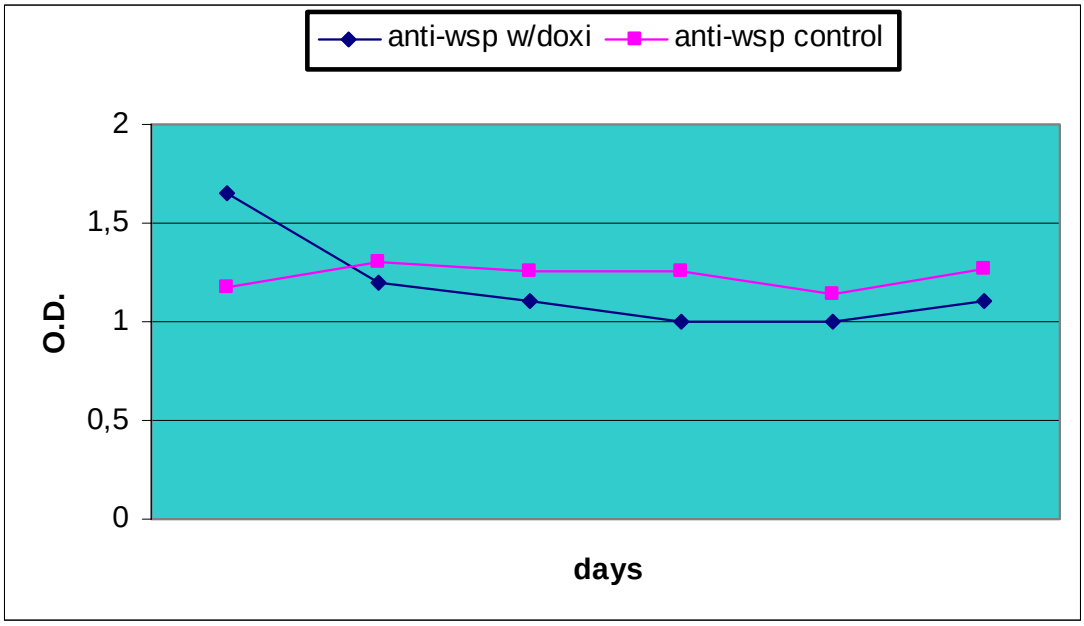

Figure 2. Anti-WSP antibody titres in *D. immitis* naturally-infected dogs treated with doxycycline: treated dogs tend to have a decrease in OD values against WSP at day 31.

dogs, according to the AHS guidelines. The following parameters were evaluated at different intervals pre- and post-adulticide therapy: thoracic radiographs; complete blood counts, biochemistry and proteinogramm; peripheral blood cytokine profiles; production of specific antibodies against *Wolbachia*. Preliminary results indicate that there was no apparent radiological improvement with antibiotic treatment. However, there was a decrease in gamma-globulin concentrations, associated with a decline in circulating anti-WSP antibodies (Figure 2). Furthermore, non-treated dogs had strong expression of IL-8 in peripheral blood as early as one week post-adulticide therapy, while in doxycycline treated animals, IL-8 production was only seen approximately 20 days following therapy and at much lower levels. These results have encouraged us to continue evaluation of the clinical benefits of antibiotic treatment in naturally infected dogs, including quantitative analyses of *Wolbachia*. In the preliminary trial, doxycycline was likely administered at too low a dose for too short a time: higher doses (20 mg kg^{-1}) given for eight weeks may very well show significant decrease in *Wolbachia*-mediated inflammatory reactions.

Given the recent and very promising developments in the use of tetracyclines for micro-macrofilaricidal therapy in human filariosis, it is hoped that similar attention will be given to canine and feline heartworm disease that could greatly benefit from alternative therapeutic strategies.

References

1. Bandi C, McCall JW, Genchi C, Corona S, Venco L, and Sacchi L. Effects of tetracycline on the filarial worms Brugia pahangi and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia*. International J Parasitol 1999;29:357–364.
2. Bandi C, Trees AJ and Brattig NW, *Wolbachia* in filarial nematodes: evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases. Vet Parasitol 2001; 98: 215–238.
3. Hoerauf A, Mand S, Adjei O, Fleisher B and Buttner DW, Depletion of *Wolbachia* endobacteria in *Onchocerca volvulus* by doxycycline and microfilaridermia after ivermectin treatment. Lancet 2001; 357: 1415–1416.
4. Hoerauf A, Mand S, Volkmann L, Buttner M, Marfo-Debrekyei Y, Taylor M, Adjei O, and Buttner DW, Doxycycline in the treatment of human onchocerciasis: kinetics of *Wolbachia* endobacteria reduction and inhibition of embryogenesis in female *Onchocerca* worms. Microb Infect, 2003a; 5: 261–273.
5. Hoerauf A, Manid S, Fischer K, Kruppa T, Marfo-Debrekyei Y, Debrah AY, Pfarr KM, Adjei O and Buttner DW, 2003b Doxycycline as a novel strategy against bancroftian .lariasis-depletion of *Wolbachia* endosymbionts from *Wuchereria bancrofti* and stop of micro- filaria production. Medical Microbiology and Immunology 192, 211–216.
6. Langworthy N, Renz A, Meckenstedr U, Henkle-Duhrsen K, Bronvoort M, Tanya V, Donnelly M and Trees AJ, Macrofilaricidal activity of tetracycline against the .larial nematode Onchocerca ochengi: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. Proc Royal Soc London Series B Biolog Sci 2000; 267:1063–1069.
7. Taylor MJ, Cross HF, Ford L, Makunde W H, Prasad GBKS, Bilo K, *Wolbachia* bacteria in filarial immunity and disease, P Immunol, 2001; 23: 401-409.
8. Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S, and Hoerauf A, Macro.laricidal activity after doxycycline treatment of *Wuchereria bancrofti*: a double-blind, randomised placebo-controlled trial. Lancet 2005; 365:2116-2121.