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HOW *WOLBACHIA / DIROFILARIA IMMITIS* INTERACT?

Laura H. Kramer, DVM, PhD, Diplomate EVPC
College of Veterinary Medicine
University of Parma
Parma, Italy

INTRODUCTION

Dirofilaria immitis, the causative agent of canine and feline heartworm disease, harbours intracellular bacteria named *Wolbachia pipientis*. Indeed, most filarial species studied so far, with very few exceptions, contain these microorganisms which are thought to play an essential role in the biology and reproductive functions of their filarial hosts. The following manuscript will focus on what is currently known about the relationship between *Wolbachia* and filarial worms and how the understanding of what *Wolbachia* actually does to/for the worm may aid researchers in finding interesting new targets for control and prevention of heartworm disease.

WHAT IS *WOLBACHIA*?

Wolbachia pipientis, the only species thus far identified in the genus, are gram-negative bacteria belonging to the order Rickettsiales. They closely resemble other bacteria belonging to the same group like *Ehrlichia* spp and *Anaplasma* spp (Bandi et al, 2001). Initial descriptions of bacterial-like structures using electron microscopy (Sacchi et al 2002) and more recent studies by immuno-histology have provided a comprehensive description of the distribution of *Wolbachia* in *D. immitis* (Bandi et al, 1999; Kramer et al., 2005) They are found throughout all the stages of the life cycle of the nematode although they occur in varying proportions between individual worms and different developmental stages (Kozek, 1977; Kozek and Figueroa, 1977; McGarry et al., 2004). In adult D*. immitis*, *Wolbachia* is predominantly found throughout the hypodermal cells of the lateral cords (Figures 1 and 2). The bacteria occur within host-derived vacuoles in variously sized discrete groups ranging from a few organisms, often clustered around hypodermal nuclei, to areas where they almost completely fill the cellular environment reminiscent of bacteriocytelike structures. In females, Wolbachia is also present in the ovaries, oocytes and developing embryonic stages within the uteri (Figure 3), whereas they have not been demonstrated in the male reproductive system (Sacchi et al., 2002). This suggests that the bacterium is vertically transmitted through the cytoplasm of the egg and not through the sperm.

HOW DOES *WOLBACHIA* INTERACT WITH ITS FILARIAL HOST?

All current evidence suggests that *Wolbachia* is a symbiont in filarial worms: i.e. the presence of the bacteria is essential for the filarial worm's survival. The phenomenon of bacterial endosymbiosis is well known in arthropods, but less so in nematodes. There are, however, several features of the relationship between *Wolbachia* and filarial worms (including *D. immitis*) that suggest its symbiotic nature: 1) in those species of filarial worms that have been identified as harbouring *Wolbachia*, all of the individuals are infected: i.e. 100% prevalence; 2) the evolution of the bacteria match that of the filarial worms, as shown by phylogenic studies; 3) the bacteria are transmitted from female to off-spring: vertical transmission can lead to the establishment of different kinds of host-symbiont relationships, the most obvious being mutualistic symbiosis where the symbiont increases its own fitness by increasing the fitness of the host that is involved in its transmission; 4) removal of *Wolbachia* (antibiotics/radiation) leads to sterility of females and eventual death of adults.

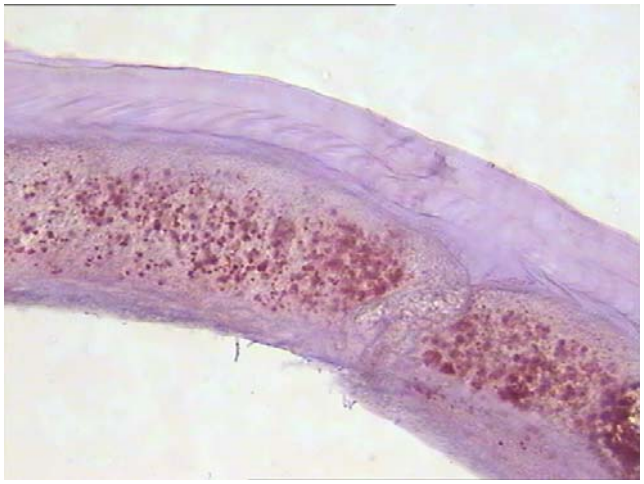

Figure 1. Cross section of an adult female *Dirofilaria immitis* stained with a polyclonal antibody against the *Wolbachia* Surface Protein (X40). Note the numerous bacteria that almost entirely fill the cell of the lateral hypodermal cord.

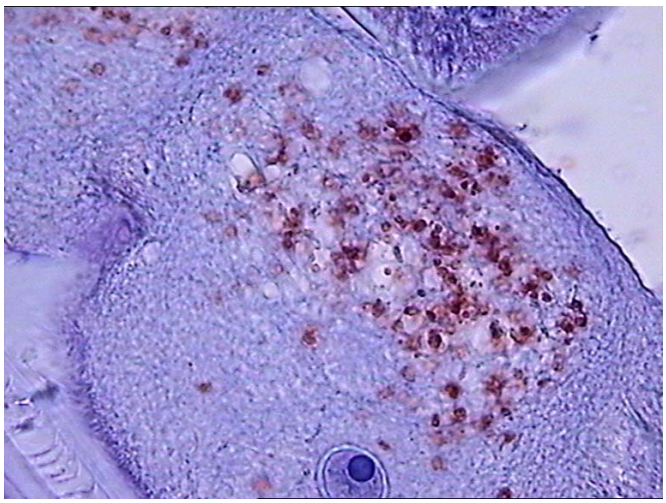

Figure 2. Cross section of an adult *Dirofilaria* male, treated as in Figure 1 (X40). The bacteria are less abundant.

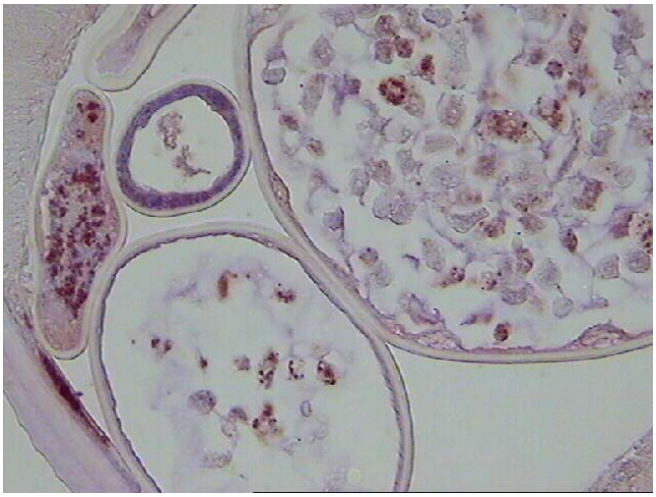

Figure 3. Cross section of an adult female *Dirofilaria immitis* treated as above. Note the reporductive system with numerous oocytes and embryos harbouring *Wolbachia* (X40).

It is still unclear however exactly what *Wolbachia* does to make it so important for its filarial host. Several hypothesis have been suggested by different researchers within the field. Quantification of bacterial numbers in different developmental stages has been studied in *Brugia malayi* (McGarry et al., 2004; Fenn and Blaxter, 2004) and have shown that the numbers of bacteria remain static in microfilariae and the mosquito-borne larval stages (L2 and L3). However, within the first week of infection of the mammalian host, bacteria numbers increase dramatically and the bacteria/worm ratio is the highest of all life-cycle stages. The rapid multiplication continues throughout L4 development, so that the major period of bacterial population growth occurs within the first month of infection of the definitive host. Infection levels are then maintained in adult males while in females, bacterial numbers increase further as the worms mature and as the ovary and embryonic larval stages become infected (McGarry et al., 2004). The changes in the dynamics of *Wolbachia* populations throughout the life cycle may therefore illustrate the points at which the symbiotic relationship is critical. The rapid increase in bacterial numbers during the period of larval and embryonic development is consistent with a role for the bacteria in these processes. The pattern of population growth would also be compatible with a role in evasion of mammalian immunity and for the long-term survival of adult worms. Interestingly, Morchon et al (2004) have recently reported that the development of a strong antibody response against experiemtnal infection of cats with *D. immitis* occurs after one-two months of infection: is it possible that this intense immune response to *Wolbachia* is characteristic of resistance to infection in this host? Bacterial population dynamics may also explain the differential activity of bacteriostatic antibiotic treatment on distinct developmental stages, in which larval and embryonic development are associated with rapidly dividing bacteria and are affected soon after antibiotic treatment, whereas the more slowly dividing populations in adults take longer to deplete and for the consequences to show. Indeed, 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 were lively and motile, exactly like thier control-dogs counterparts. Furthermore, there was no difference in microfilarial concentration between treated and control dogs. However, when uterine content of these worms was examined, there was a dramatic decrease in the number of pretzels and stretched microfilariae, indicating that bacteriostatic antibiotic treatment was able to block embryogenisis.

Perhaps the most important recent discovery concerning *Wolbachia* in filarial nematodes is the completion of the entire genome sequencing and annotation of the metabolic pathways of the *Wolbachia* from *Brugia malayi* (Foster et al, 2005). The genome is very small and this is consistent with endosymbionts: evolution of symbiosis has greatly reduced those things the bacteria have to “make” for it’s survival. The fialrial host likely supplies the bacteria with amino acids necessary for growth and replication (it seems that *Wolbachia* doesn’t synthetize any except one for its peptidoglycan wall). On the other hand, *Wolbachia* does synthetize several important molecules that may be essential for its filarial host. Candidates include: 1) glutathione: glutathione biosynthesis genes may be a source of glutathione for the protection of the host nematode from oxidative stress or immunological effector molecules; 2) haeme: haeme from *Wolbachia* could be vital to worm embryogenesis as there is evidence that moulting and reproduction are controlled by hormones whose synthesis requires haeme. Depletion of *Wolbachia* might therefore halt production of these hormones and block embryogenesis. Studies of the genome have also shown what *Wolbachia* does not make, namely lipopolysaccharides, an important component of the outer wall of most gram-negative bacteria. Indeed, this discovery has lead to re-thinking among researchers who had suggested in the past that the pro-inflammatory properties of *Wolbachia* were likely due to an endotoxic activity (Taylor et al, 2000; see “The role of *Wolbachia* in inflammatory...” in this volume).

CONCLUSIONS

Wolbachia is essential for its filarial host, including *D. immitis*. Evolutionary studies confirm that filarial worms have been harboring *Wolbachia* for tens of millions of years. During this time, the bacteria have learned to supply needed metabolites to the worm during moulting and embryonal development and the worm, in turn, has garanteed the bacteria’s survival and transmission. It is indeed a “one hand washing the other” situation that may, however, be the key to novel strategies for the control/treatment of filarial infection, including canine and feline heartwrom disease.

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. Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, Bhattacharyya A, Kapatral V, Kumar S, Posfai J, Vincze T, Ingram J, Moran L, Lapidus A, Omelchenko M, Kyrpides N, Ghedin E, Wang S, Goltsman E, Joukov V, Ostrovskaya O,Tsukerman K, Mazur M, Comb D, Koonin E and Slatko B, The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. PLoS Biology 2005; 3: 121-129
4. Kozek WJ, Transovarially-transmitted intracellular microorganisms in adult and larval stages of *Brugia malayi*. J Parasitol 1977; 63: 992–1000.
5. Kozek WJ and Figueroa MH, Intracytoplasmic bacteria in *Onchocerca volvulus*. Am J Trop Med Hyg 1977; 26: 663–678.
6. Kramer LH, Tamarozzi F, Morchon R, Lopez-Belmonte J, Marcos- Atxutegi C, Martin-Pacho R, and Simon F, Immune response to and tissue localization of the *Wolbachia* surface protein (WSP) in dogs with natural heartworm (*Dirofilaria immitis*) infection. Vet Immunol Immunopathol 2005; 106 (3-4): 303-308.
7. McGarry HF, Egerton G and Taylor MJ, Population dynamics of *Wolbachi*a bacterial endosymbionts in *Brugia malayi*. Mol Biochem Parasitol 2004; 135: 57–67.
8. Morchon R, Ferreira A C, Martin-Pacho J, Montoya A, Mortarino M, Genchi C and Simon F, Specific IgG antibody response against antigens of Dirofilaria immitis and its *Wolbachia* endosymbiont bacterium in cats with natural and experimental infections. Vet Parasit 2004; 125: 313-321.
9. Sacchi L, Corona S, Casiraghi M and Bandi C, Does fertilization in the filarial nematode *Dirofilaria immitis* occur through endocytosis of spermatozoa? Parasitology 2002; 124: 87–95.
10. Taylor MJ, Cross HF and Bilo K. Inflammatory responses induced by the filarial nematode *Brugia malayi* are mediated by lipopolysaccharide- like activity from endosymbiotic *Wolbachia* bacteria. J of Exp Med 2000;191: 1429–1436.