

Worm warfare in West Africa

By Joe Neary (6th Year, Cambridge)

Joe Neary, who is due to graduate from Cambridge Vet School this summer, spent his eight-week BSc project in Cameroon, part of which was funded by the Harry Steele-Bodger Memorial Fund.

Filarial nematodes are major pathogens that are responsible for debilitating diseases in human populations of the tropics (river blindness, caused by *Onchocerca volvulus*, and lymphatic filariasis or elephantiasis, caused by *Wuchereria bancrofti*) and in animals (canine heartworm, caused by *Dirofilaria immitis*).

For my final-year elective project I was determined to investigate something that I thought to be particularly interesting and worthwhile. River blindness infects an estimated 37 million people, with 90 million at risk of infection in Africa. The disease is known as river blindness because of its high prevalence in villages located along fast-flowing rivers where the vector, the female *Siumlium* blackfly, breeds. Symptoms may include visual impairment and blindness, itching sufficient to cause self-mutilation and musculoskeletal pain. Currently, no drug safe enough for mass chemotherapy is available to kill adult worms, which can live for approximately 10 years.

Professor Sandy Trees' research group at the Liverpool School of Tropical Medicine (LSTM) is investigating *Onchocerca* species infections in West African cattle. Cattle offer the most practicable, analogous host-parasite model system in the study of river blindness. Intrigued by the application of veterinary training in the study of a human disease, I contacted Professor Trees to ask if he would consider supervising my elective project. I was pleasantly surprised by his enthusiastic response.

Many filarial nematodes contain an endosymbiotic, *Rickettsia*-like

bacterium of the genus *Wolbachia*. Previous studies have suggested that worm survival may depend on this bacterium. West African cattle are commonly co-infected with four *Onchocerca* species: two of which are *Wolbachia* positive (*O gutturosa* and *O ochengi*), and two are of unknown *Wolbachia* status (*O dukei* and *O armillata*). *O Armillata*, an abundant parasite of African cattle that has received little attention, is a primitive species that may lack *Wolbachia*. The objectives of my study were to determine if *O armillata* carries *Wolbachia* species and to provide preliminary descriptions of the host inflammatory cell environment around *O armillata*. The findings may support or refute the hypothesis that a prime contribution of *Wolbachia* is to permit long-term survival and reproduction of certain *Onchocerca* species (which include *O volvulus* in humans). If true, *Wolbachia* may provide a suitable therapeutic target in the treatment of and/or vaccination against river blindness.

My most daunting task was to raise sufficient funding to cover the costs of the eight-week project. Thanks to generous support from the Wellcome Trust Vacation Scholarship, the British Veterinary Association (BVA) Harry Steele-Bodger Memorial Fund, the British Cattle Veterinary Association (BCVA) Student Clinical Research Grant, an Intervet Student Vacation Bursary, a Pfizer Vacation Study Grant and the Cambridge Veterinary School Jowett Fund, I was able to achieve this.

Off to Africa

In July, I flew to the capital of Cameroon, Yaoundé, to begin my three-week stay in beautiful West Africa. The next day I caught the overnight train to Ngaoundéré, in the highlands of North Cameroon, where I was to start my project. The suppressive combination

of heat and insect repellent was enough to repel even the thirstiest of mosquitoes from the sleeping cabin. Instead, they joined me and the strong smelling cassava outside in the passageway for the night.

The following day I started my project work at the abattoir where approximately 100 cattle are slaughtered each morning. I had been forewarned that the abattoir was not too dissimilar from Delacroix's paintings of hell. It consisted of a roof suspended above an area of concrete roughly the size of a tennis court. There was a drainage channel running along the middle and an entrance for the cattle to be run through on one side. Occasionally, slaughtermen would scream in alarm as an animal slipped the rope from its horns and jumped over several freshly skinned carcasses before capture. At the smell and sound of death, bulls would sit down 20 metres from the abattoir, refusing to enter. Over 10 men pulling on ropes would be required to drag the animal in through the abattoir opening. And then there was me – jumping out of the way of carcasses and apologising profusely, 'Pardon! Pardon! Je m'excuse!', to busy men as I followed a potential source of samples through the organised chaos.

At the end of the spectacle the meat would be loaded onto the waiting trucks and, although prohibited, on to the back of motorbike taxis, quite spectacularly! Armed with my samples consisting of the aorta (containing adult worms in the artery wall) and skin (containing microfilariae), I would set off to the laboratory in a nearby village. Here, I would record the gross pathology and worm nodule prevalence within the aorta wall. The motility and viability of the adult worms was scored. Snips from the superficial epidermis were incubated in tissue culture solution and the emerging microfilariae differentiated into their species and counted under a microscope. Without help from David and Henrietta I could not have done this. Thankfully, I no longer dream of counting micro-

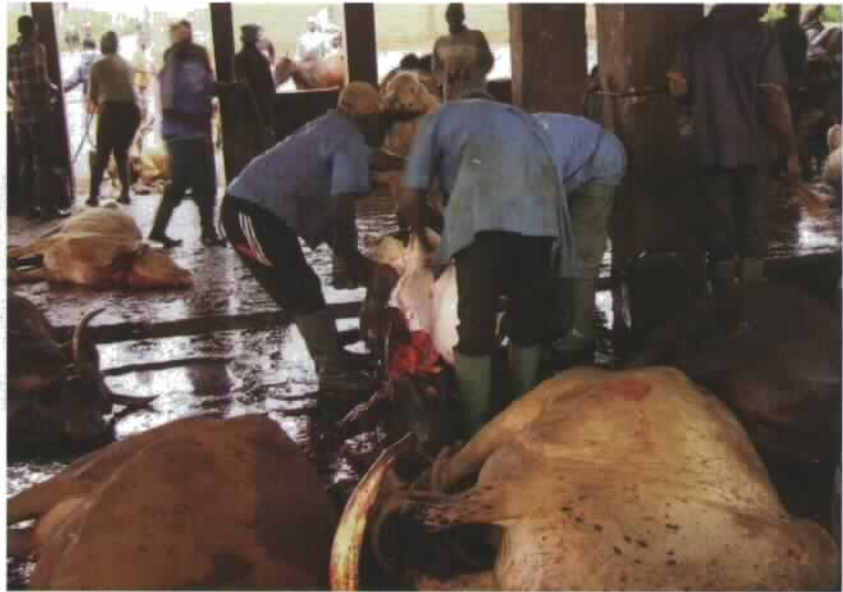
filariae in my sleep! Samples of the aorta wall were preserved in formalin for histopathology and adult worms frozen for PCR analysis to be performed when back at the LSTM.

Intermittent electricity supply

However, the laboratory's electricity supply would often cease without warning due to the installation of a water pump in a nearby village. With no power for the microscopes and Shabba Ranks rudely cut off in mid-song (the lab had a limited collection of reggae CDs), there was nothing to do but wait nervously under the shade of a tree hoping that my samples, slowly warming in the freezer, were not perspiring as much as me! There was no water supply either, but at least that was consistent!

When not busy on my own project I would help/hinder another exciting project being undertaken by PhD student Rowena Barty on *Onchocerca onchengi* infection in cattle. Otherwise, I would climb Mount Ngaoundèrè (known to one local youngster with football skills to match his imagination as 'le mamelon') or go down into the town and nearby football pitches.

Back at the LSTM the presence of *Wolbachia* in *O armillata* was confirmed by a specific anti-*Wolbachia* surface protein antibody detected using a peroxidase conjugate (for immunohistochemistry), and PCR techniques were used to detect *Wolbachia*-specific sequences within DNA extracts from frozen worms.



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Tissue sections stained with haematoxylin and eosin showed the host cell response to be predominated by macrophages and fibroblasts. This is unusual for a *Wolbachia* positive *Onchocerca* species where the host response is typically characterised by granulocytes. I suspect that *O Armillata* is a motile species that is less dependent on a local neutrophilia acting as an 'immunological blockade' to protect against eosinophil infiltration and degranulation. However, perhaps *Wolbachia* are required for such motility to occur. *Wolbachia* may indeed provide a suitable drug target in the treatment of river blindness, but for reasons as yet not fully understood.

O Armillata was highly prevalent (50 of 54 cattle examined) and

the severity of the pathological lesions may have implications for animal production in the tropics. Although no obvious signs of illness are reported in cattle infected with *O Armillata*, the severity of the pathological lesions that I found must reduce the efficiency of blood flow through the aortic arch. In combination with the multiple parasitic infections that many cattle have, the cumulative effects may be significant.

Excitement of research

This experience has strengthened my belief that research (and a final-year elective project) can be exciting. I have had the privilege of learning skills, acquiring knowledge and making international friendships with many amazing people. I encourage students to take advantage of the generous funding available to undertake such projects.

As Bob Marley told me on a daily basis while in Cameroon, 'Don't gain the world and lose your soul, wisdom is better than silver or gold'. Special thanks to my supervisor, Professor Sandy Trees, for giving me the opportunity to participate in his research team, Dr Ben Makepeace for his time and guidance, the Harry Steele-Bodger Memorial Fund Trustees and my other sponsors for their generous support.



Me, Henrietta and David in the lab taking time out from counting microfilariae during a power cut