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SHORT COMMUNICATION

A preliminary investigation of serological tools for the detection of *Onchocerca lupi* infection in dogs

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Abstract *Onchocerca lupi* is a neglected filarioid causing nodular lesions associated with acute or chronic ocular disease in dogs. Despite the recent appraisal of its zoonotic potential, human cases are increasingly reported in the Old and New Worlds. Therefore, the development of accurate tools for the rapid diagnosis of *O. lupi* infections in dogs is becoming a priority. In this study, we conducted a preliminary investigation aimed at evaluating the usefulness of a commercially available ELISA test for the detection of *O. lupi* antigens in canine sera. The potential use of this tool for larger epidemiological studies of canine onchocerciasis is discussed.

Keywords *Onchocerca lupi* · Serology · Dog · Cross-reactivity · Og4C3 · Lymphatic filariasis

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Introduction

Filarioids (Spirurida, Onchocercidae) are amongst the most important vector-borne helminths of public health concern (Orihel and Eberhard 1998). While knowledge of the transmission and control of human infections by the canine filarioids *Dirofilaria immitis* and *Dirofilaria repens* is extensive (Pampiglione et al. 2009), data on the epidemiology, diagnosis and zoonotic potential of *Onchocerca lupi* is limited. This neglected spirurid has been recently detected in dogs from Europe (Hungary, Greece, Germany and Portugal) (Otranto et al. 2013a) as well as in carnivores from the USA (Labelle et al. 2013). In the canine host, onchocercosis is often asymptomatic and seldom results in mild or severe ocular lesions (Sréter and Széll 2008). Interestingly, cases of human onchocercosis by *O. lupi* have been reported in young patients from Turkey, Iran, Tunisia and the USA (Otranto et al. 2011; Eberhard et al. 2013; Mowlavi et al. 2013). In humans, the clinical signs may vary widely and include disfiguring nodules on the conjunctiva associated with variable degrees of inflammation around the ocular globe (Otranto et al. 2011; Mowlavi et al. 2013) as well as the development of soft masses into the cervical channel (Eberhard et al. 2013).

Traditionally, the diagnosis of canine onchocercosis consists of the detection of dermal microfilariae from the skin and of adult nematodes from excised nodules, followed by their morphological and/or molecular identification (Sréter and Széll 2008; Otranto et al. 2013a). However, diagnosis usually requires time-consuming and invasive procedures as well as considerable technical skills for the isolation and identification of the parasites (Otranto et al. 2013a, b). Moreover, pre-patent infections or infections with small numbers of microfilariae can lead to false-negative results, thus representing an obstacle to timely interventions (Otranto et al. 2013a, b). Therefore, in order to achieve a rapid and reliable diagnosis of canine and human onchocercosis, the development of serological

methods based on the detection of parasite antigens or specific host antibodies against the parasite is becoming a priority.

In previous studies, aiming at developing novel diagnostic methods for the detection of emerging life-threatening diseases caused by filarioids, cross-reactions between antibodies and heterologous antigens have been successfully exploited (More and Copeman 1990). One example is represented by Og4C3 (Cellabs®, Sydney, Australia), an enzyme-linked immunosorbent assay (ELISA), developed with a monoclonal IgM antibody against non-phosphorylcholine and heat-stable circulating proteins secreted by *Onchocerca gibsoni*. This tool had already been proven useful for the detection of excreted/secreted (E/S) antigens of the agent of human lymphatic filariasis (*Wuchereria bancrofti*) (More and Copeman 1990; Chanteau et al. 1994) and could be further exploited for the detection of other species of filarioid parasites in *O. gibsoni*- and *W. bancrofti*-free areas. In the present study, we performed a preliminary investigation of the potential use of this commercially available ELISA for the detection of *O. lupi* antigens in sera from infected dogs.

Materials and methods

The study was conducted according to the principles of Good Clinical Practice (VICH GL9 GCP, 2000 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/ WC500004343.pdf). Dogs infected by *O. lupi* (n=6) were available from a previous study (Otranto et al. 2013a). Briefly, all animals were positive for microfilariae at the skin snip test, performed with biopsy punch on the inter-ocular frontal area of the head. Samples were soaked at 37 °C in saline solution for 1 h, sediments (20 μl) were observed under a light microscope and microfilariae were counted (Table 1). Finally, they were morphologically and molecularly identified (Otranto et al. 2013a, b). Blood samples were collected from the brachial vein in serum-separating tubes, allowed to clot at room temperature and centrifuged at 1,700×g for 10 min. Sera were separated and stored at −20 °C. In addition, sera from dogs solely infected by *Cercopithifilaria bainae* (n=12), *Acanthocheilonema reconditum* (n=3) or *D. repens* (n=5) were included in the study as well as five samples collected from negative control dogs. All samples were processed in duplicate according to the Og4C3 manufacturer's instructions at the laboratories of the WHO Collaborating Centre for the Control of Lymphatic Filariasis, Soil-Transmitted Helminths and Other Neglected Tropical Diseases of James Cook University (Cairns, Queensland, Australia), as previously described (More and Copeman 1990; Chanteau et al. 1994).

Table 1 *Onchocerca lupi* sera tested: mean number of microfilariae (mfs) in 20 μl of skin sediment and results at spectrophotometry (optical density average at 405 nm). Cut-off: 0.199 OD

Sample	Mean number of mfs	Og4C3 ELISA
Dog 1	26.2	Reactive (0.255)
Dog 2	38.4	Reactive (0.876)
Dog 3	47.1	Non-reactive
Dog 4	13.4	Non-reactive
Dog 5	23.7	Non-reactive
Dog 6	45.8	Reactive (0.414)

Results and discussion

Circulating antigens of *O. lupi* were detected in three out of six sera collected from skin snip-positive animals, while those from dogs infected by other filarioids (i.e. *C. bainae*, *A. reconditum* and *D. repens*) and uninfected control animals remained negative (Table 1).

Results from the present study indicate that the Og4C3 kit successfully detected three out of six of the known infections by *O. lupi*, and, albeit preliminary, this outcome should be taken into account for future investigations of the application of serological tools for epidemiological studies of canine onchocercosis on a larger scale. These results need to be carefully evaluated since canine sera positive for other filarial nematodes, such as *D. immitis*, whose distribution may overlap that of *O. lupi* (Otranto et al. 2013c), were not tested. Also, the small number of sera from dogs infected by *O. lupi* represents an obvious limitation of this study. However, the lack of parasite-associated clinical signs in infected dogs, along with the difficulties in achieving a specific diagnosis through the skin snip test are limiting factors which affect the collection of large numbers of positive sera. Despite this limitation, the Og4C3 kit yielded positive results only for sera from *O. lupi* infected animals, while those infected by other filarioids (i.e. *C. bainae*, *A. reconditum*, *D. repens*) scored negative. This result suggests that the *O. lupi* antigen detected by the Og4C3 shares similar epitopes with antigens from *O. gibsoni*, *W. bancrofti* and *D. immitis* (More and Copeman 1990). In particular, the specific antibodies included in the Og4C3 kit bind protein and carbohydrate moieties of *O. gibsoni*; these moieties are localized at the junction of the cuticle and the hypodermis and on gut cell membranes of the male nematode and on microfilariae embryos (More and Copeman 1990). However, in *W. bancrofti*, the same antibodies have been shown to react with E/S antigens elicited by the adult worms (Chanteau et al. 1994). Further studies are needed to establish the relationships between the number of microfilariae in the skin and the antigenaemia, which will ultimately allow a precise estimation of the sensitivity and specificity of the Og4C3 test for the detection of *O. lupi*. In any case, the identification of the specific *O. lupi* antigen(s) binding to the specific antibodies remains a priority and the use of other well-studied filarioids (e.g. *D. repens*, *D. immitis*,

Onchocerca volvulus, *O. gibsoni* and *W. bancrofti*), as references, will be pivotal to assess the sensitivity and specificity of modified ELISA tests for the diagnosis of *O. lupi* infection. Alternatively, the potential use of paramyosin, a component of nematode muscles, could be investigated. Indeed, given the highly immunogenic properties of this protein, as well as its conserved structure across nematode species, this molecule has been already exploited for the design of diagnostic and chemoprophylaxis devices of other parasitic diseases (e.g. schistosomiasis) (Gobert and McManus 2005).

In conclusion, we have demonstrated that the Og4C3 test may represent a potential valid diagnostic tool for the detection of *O. lupi* in dogs. Future studies aimed at optimizing the sensitivity and specificity of this kit for its application to large-scale epidemiological surveys will contribute towards the definition of the real prevalence and incidence of the infection in endemic areas.

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Conflict of interests The authors declare that they have no competing interests.

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