

Short note

## IgG response against infective larvae of *Dirofilaria immitis* in experimentally infected cats

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**Abstract** – Somatic antigens from third stage larvae of *Dirofilaria immitis* (SL3) were used to detect IgG response against heartworm infection in 8 experimentally infected cats. A moderate specific anti-SL3 IgG response was found one month post-infection. Afterwards, antibodies decreased reaching a basal level 4 months post-infection and remained at this level until the end of the study, 6 months post-infection. Western blot analysis showed specific recognition of polypeptides of 79, 73, 60, 52, 40 and 39 kDa by sera from infected cats 1 month post-infection, but not by sera taken prior to the infection. The low antigenicity of the SL3 antigen in the cat should allow the parasite to escape the host's immune response.

**cat / heartworm infection / L3 antigens / IgG response**

**Résumé** – Réponse en IgG contre les larves infectieuses de *Dirofilaria immitis* chez des chats expérimentalement infectés. Des antigènes somatiques provenant de larves du troisième stade de *Dirofilaria immitis* (SL3) ont été utilisés afin de détecter une réponse en IgG contre une infection à *Dirofilaria immitis* chez huit chats expérimentalement infectés. Une réponse modérée des IgG spécifiques anti-L3 a été détectée un mois après infection. Ensuite, les anticorps ont diminué jusqu'à atteindre un niveau de base 4 mois après infection, et sont restés à ce niveau jusqu'à la fin de l'étude, 6 mois après infection. Une analyse en Western blot a montré une reconnaissance spécifique de polypeptides de 79, 73, 60, 52, 40 et 39 kDa par les sérums de chats infectés, un mois après infection, mais pas par des sérums prélevés un mois avant infection. La faible antigénicité de l'antigène SL3 chez le chat devrait permettre au parasite d'échapper à la réponse immunitaire de son hôte.

**chat / infection à *Dirofilaria immitis* / antigène L3 / réponse IgG**

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## 1. INTRODUCTION

Feline heartworm (*Dirofilaria immitis*) infection has been diagnosed with increasing frequency in the last years in areas where the disease is endemic in dogs. In cats, the infection is characterised by a low worm burden (often 1–2 worms); microfilaremia is transitory and of low intensity and usually naturally infected cats are amicrofilaremic. The evolution of the disease is variable being generally asymptomatic, although sudden death of asymptomatic cats is relatively frequent [1, 5]. In cats, a strong IgG response to adult worm antigens is observed and different polypeptides ranging between 19 and 40 kDa are recognized by Western blot [7]. This response is detectable quite early post-infection (2–3 months) [8]. No data are however available on the immune response to third-stage larvae in cats. Because the survival of the infective stage is crucial for the establishment of the infection, preliminary data of the immune response against L3-somatic antigens in *D. immitis* experimentally-infected cats are presented.

## 2. MATERIALS AND METHODS

*D. immitis* infective larvae (L3) were obtained from experimentally infected *Aedes aegypti* as described by McCall [4].

Eight cats were infected by subcutaneous injection of the 30 L3/cat in the lateral region of the neck. Blood samples were obtained prior to infection (time 0) from only 3 cats, and 1, 2, 4 and 6 months post-infection from all of the 8 cats. After seven months post-infection, all cats were found to be infected with 1 to 4 worms.

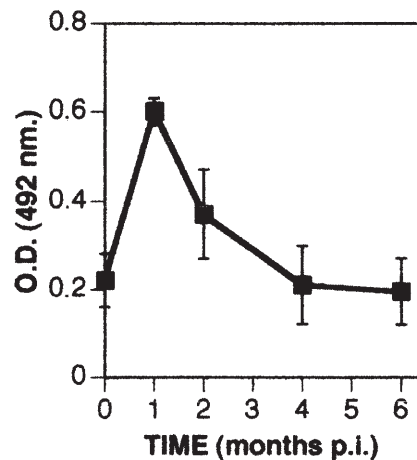
Somatic L3 antigen (SL3) preparation was performed as follows. Infective larvae were sonicated by 4 cycles of 70 kHz (1 min each) at 4 °C and centrifuged at 10 000 g for 20 min. Most of the supernatant was discarded and the resuspended pellet was concentrated by ultrafiltration at 6 000 g. The protein concentration was adjusted at 0.5 µg·µL<sup>-1</sup> and stored at 4 °C until use.

ELISA and Western blot analyses were performed as described by Prieto et al. [7] with some modifications. ELISA polystyrene plates were coated with SL3 antigen at a concentration of 0.5 µg·µL<sup>-1</sup>. Sera were employed at a single dilution of 1:200 or at serial dilutions of 1:50, 1:100, 1:200 and 1:400, and anti-IgG-peroxidase at 1:4000 dilution. The cut-off point (OD 0.462) was obtained as the OD arithmetic means plus three standard deviations for 43 sera from clinically healthy cats, serologically negative for antibodies against *D. immitis* by a commercially available in-clinic test (Heska™ Solo Step™ FH, Heska Corporation, Fort Collins, Colorado, USA), confirmed by an experimental ELISA with somatic and excretory/secretory antigens [7]. In Western blot, pooled sera were employed at a 1:50 dilution and the anti-IgG-peroxidase at a 1:500 dilution.

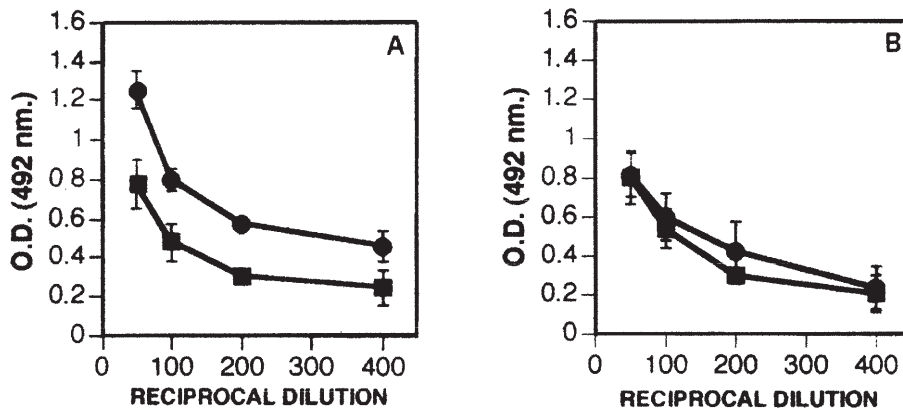
The statistical analysis was performed by one-way ANOVA and a 5% level of significance ( $p \leq 0.05$ ) was used to assess statistical differences.

## 3. RESULTS

Specific anti-SL3 IgG was detected at moderate levels (OD = 0.6) only 1 month



**Figure 1.** Antibody response (mean ODs) to SL3 antigens in 8 experimentally infected cats.

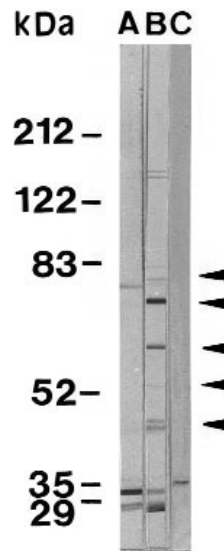


**Figure 2.** Mean OD obtained by ELISA analysis with the SL3 antigen from sera from 3 experimentally infected cats obtained prior to infection and from 8 cats 1 and 2 months post-infection. **A.** ELISA with sera from cats prior to infection (■) and 1 month post-infection (●). **B.** ELISA with sera from cats prior to infection (■) and 2 months post-infection (●). Standard deviation bars are shown.

post-infection; afterwards the antibodies decreased, reaching a basal level 4 months post-infection and remaining at this level until the end of the study (Fig. 1). A comparison of the ODs of sera from 1 month post-infection and time 0 indicated that all of the tested dilutions were significantly different. No differences were found between time 0 and 2 months post-infection (Fig. 2). Western blot analysis showed specific recognition of polypeptides of 79, 73, 60, 52, 40 and 39 kDa by sera from infected cats 1 month post-infection, but not by sera taken prior to infection (time 0) and by serum from a cat infected with *Ancylostoma tubaeforme* (Fig. 3).

#### 4. DISCUSSION

Our results, though obtained from a low number of experimentally infected cats, showed that the immune response against L3-somatic antigens in cats is weak, of short duration and able to recognize a low number of antigens. Previously, a strong IgG response against adult worm antigens was



**Figure 3.** Western blot analysis of the SL3 antigen with a pool of sera from 3 cats prior to infection (**A**), a pool of sera from 3 cats 1 month post-infection (**B**) and serum from a cat infected with *Ancylostoma tubaeforme*.

found from the 2nd–3rd month post-infection on, probably as a consequence of common antigens shared both by 4th stage larvae, preadult and adult worms [7, 8].

In areas endemic for canine heartworm infection, the seroprevalence of IgG antibodies against the parasite is quite high in cats (44%) with respiratory and gastrointestinal signs [9]. Furthermore, in an extensive survey involving more than 2 000 cats, who were mostly asymptomatic, Miller et al. [6] found that the overall risk of exposure to heartworm infection was about 12%, ranging from 5–33%. It is thought however that many cats spontaneously recover and no clinical signs of infection are observed. Because the immune response against the infective stage seems to be quite low and of short duration, the parasite can probably develop to the 4th larval stage. The strong immune response detectable at the 4th month post-infection [7, 8], is likely to interfere with the development of the parasite at the adult stage. The low level of anti-SL3 IgG antibody and the short period in which they are detectable should be due to the short life span of this stage (3–9 days) [3]. Furthermore, our results seem to confirm the observations of Ibrahim et al. [2]. In this study, both *in vitro* and *in vivo* *D. immitis* L3 were found to shed many surface antigens (mostly of 6 and 35 kDa) which were not replaced. This loss of surface peptides resulted in a reduction in the antigenic potential of infective larvae. We therefore conclude that shedding of surface peptides and reducing surface antigenicity may represent mechanisms by which *D. immitis* infective larvae evade the host immune attack. Because the immune response to L3 larvae seems to be critical, it is possible to speculate that antigens from the L4 larvae may be better able to induce protective immunity than extracts from the L3 stage. The study of immune response to L4 extracts should validate this hypothesis.

## ACKNOWLEDGEMENTS

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