

A COMPARISON OF TWO ANTIGEN-DETECTION ELISA FOR DETECTING INFECTION OF *DIROFILARIA IMMITIS* IN DOGS

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Summary :

A survey on 87 dogs necropsied in the Townsville region revealed 34 (39 %) were infected with *Dirofilaria immitis*. Infected dogs had an average of 6.1 adult worms in the heart and associated blood vessels. The VetRED assay detected 23 of the 34 infected dogs (sensitivity 65 %) and the Og4C3 ELISA detected 27 (sensitivity 80 %). Sensitivity of the VetRED and Og4C3 ELISA increased to 88 and 94 % respectively in dogs with three or more worms. Both tests detected correctly all uninfected dogs. Despite the higher accuracy of the Og4C3 ELISA, compared to the VetRED assay, it is unlikely to be used widely as a field test for heartworm unless it can be modified from its present plate ELISA format which takes 4 hours, into one which is more rapid and convenient. However, as a reference ELISA, it may well be worthwhile in situations which require considerable accuracy for detecting *D. immitis* infection.

KEY WORDS : *Dirofilaria immitis*, VetRED, Og4C3ELISA, dog.

Résumé :

COMPARAISON DE DEUX TESTS ELISA DANS LA DÉTECTION DE *DIROFILARIA IMMITIS* CHEZ LE CHIEN
L'étude de 87 chiens autopsiés dans la région de Townsville a révélé que 34 (39 %) étaient infectés par *Dirofilaria immitis*. Les chiens infectés avaient en moyenne 6,1 vers adultes dans l'appareil cardiovasculaire. Le test VetRED a détecté 23 des 34 chiens infectés (sensibilité de 65 %) et l'ELISA Og4C3 27 (sensibilité de 80 %). La sensibilité des VetRED et ELISA Og4C3 a atteint respectivement 88 et 94 % chez les chiens porteurs de trois vers ou plus. Les deux tests ont correctement détecté les chiens non infectés. Malgré la plus grande sensibilité de l'ELISA Og4C3 par rapport à celle du VetRED, il est difficile d'y recourir largement en routine pour les vers cardiovasculaires, à moins d'en modifier la forme actuelle — qui demande 4 heures — en un test plus rapide. Cependant, comme ELISA de référence, il peut être fort utile dans des situations qui demandent une grande sensibilité dans la détection d'infections à *D. immitis*.

MOTS CLÉS : *Dirofilaria immitis*, VetRED, Og4C3 ELISA, chien.

INTRODUCTION

The Og4C3 ELISA has been shown to be highly sensitive and specific for detecting *Wuchereria bancrofti* infection in humans (More & Copeman, 1990). Because it also detects the presence of *Dirofilaria immitis* in dogs, this survey was conducted to compare its accuracy with that of the widely used VetRED™ Canine Heartworm Antigen Test Kit (Agen Biomedical, Brisbane, Australia).

MATERIALS AND METHODS

The survey was conducted on 87 dogs at the Townsville Animal Refuge (NQSPCA, Bohle, Townsville) between May 1993 and July 1994.

After the dogs were euthanased with an overdose of sodium pentobarbitone, axillary arterial blood was collected into heparinised and non-heparinised vacutainer bottles. The chest was opened and the heart and lungs removed. By careful dissection of the caudal and cranial vena cavae, right heart and pulmonary artery, all *D. immitis* were removed, washed in PBS and stored on ice for transport to the laboratory. Serum was decanted from clotted blood after 1 h at room temperature, centrifuged at 1,500 g and stored at -70°C until used. Heparinised blood was used for estimation of the number of microfilariae and for testing with the VetRED™ test on the same day at the laboratory. For detection of microfilariae, 50 µL of blood was placed on a haemocytometer slide, examined under a light microscope at 40 × magnification and the number counted. This was repeated three times, values were expressed as microfilariae per mL and a mean ± standard deviation calculated.

In blood samples where no microfilariae were evident, blood was filtered using a 5 µm millipore filter (Millipore Filter Corp, Bedford, USA). The VetRED™ test was also performed according to the manufacturer's instructions, and the Og4C3 ELISA was used as des-

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cribed by More & Copeman (1990). Statistical analysis of data was performed using the Student t test. Quantitation of results for OD with the Og4C3 ELISA was achieved by fitting a multiple regression line to the \log_{10} -transformed standard curve.

RESULTS

Adult *D. immitis* were found in 34 (39 %) of the 87 dogs. Infected dogs had an average of 6.1 worms per dog (range 1 to 82). The results from the necropsy are tabulated with results of the VetRED™ test and Og4C3 ELISA in Table I. Of the 34 dogs with heartworm in the heart or pulmonary artery, 12 (35 %) were denoted as « occult dogs », as they were negative on examination for microfilariae under the microscope and with millipore filtration of blood (denoted as occult' dogs). The VetRED™ test detected two of these occult dogs and the Og4C3 ELISA five. Worms in nine of the dogs with occult infection were single sex. The other three dogs had 15, 6 and 8 mature males and 16, 2 and 1 mature females respectively.

Using the Student *t* test, there was a weak but significant positive correlation between the number of adult *D. immitis* present and the number of microfilariae in peripheral blood ($r^2 = 0.489$; $F_{1,84} = 78.44$; $p < 0.005$); a weak but significant correlation between antigen level in serum samples measured with the Og4C3 ELISA and microfilariae ($r^2 = 0.381$; $F_{1,87} = 50.44$; $p < 0.005$), and a weak but significant correlation between the number

of adult *D. immitis* and antigen levels in serum samples ($r^2 = 0.648$, $F_{1,87} = 151.06$, $p < 0.005$) (Fig. 1).

When results of the VetRED™ assay are compared to those of the Og4C3 assay, it is apparent that the VetRED™ assay has reduced sensitivity compared with the Og4C3 assay when adult parasite numbers are below a critical three adult worms (data not shown). There is still, however, a good correlation between the number of mature *D. immitis* and the resultant positive test using the VetRED™ assay ($r^2 = 0.557$, $F_{1,84} = 103.4$). In the total population, the VetRED™ had a specificity of 100 %, correctly identifying 53/53 uninfected dogs. Sensitivity of VetRED™ was poorer, with the assay detecting 22/34 infected dogs (64.7 %). In dogs infected with three or more mature worms, VetRED™ detected 22/25 dogs (sensitivity of 88 %). A weak positive correlation existed between the number of circulating microfilariae and a positive test using the VetRED™ assay ($r^2 = 0.399$, $F_{1,84} = 54.6$). One case, in which the dog had four worms (one male and three females) and a mean count of 20,000 microfilariae/mL, was positive using the VetRED™ assay, while another dog with eight worms (six males and two females) with no circulating microfilariae, was negative. The Og4C3 ELISA accurately detected infection in both these cases, and was also capable of detecting all microfilaraemic cases. The limit of detection by the Og4C3 assay was in a dog with six male and two female *D. immitis* and no circulating mf, which is equivalent to the sensitivity of the VetRED™ assay. However, in the presence of circulating microfilariae, the Og4C3 assay showed superiority. In another case,

| | Test | Worms present (n = 34) | Worms absent (n = 53) |
|---------------|---------------|------------------------|-----------------------|
| Test positive | Microfilariae | 22 | 0 |
| | VetRED™ | 22 | 0 |
| | Og4C3 | 27 | 0 |
| Test negative | Microfilariae | 12 | 53 |
| | VetRED™ | 12 | 53 |
| | Og4C3 | 7 | 53 |

Sensitivity: VetRED™ = 65 %; Og4C3 ELISA = 80 %; mf detection (smear + Difil) = 65 %.

Specificity: VetRED™ = 100 %; Og4C3 ELISA = 100 %; mf detection (smear + Difil) = 100 %.

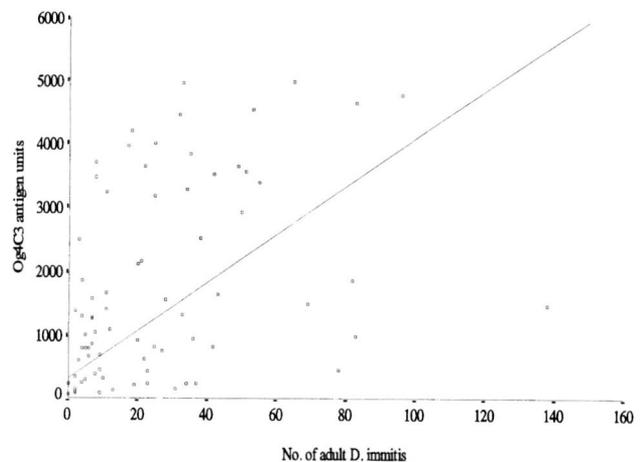


Fig. 1. – Scatterplot showing a weak positive correlation between number of *Dirofilaria immitis* present in dog hearts and associated pulmonary arteries at *post-mortem* and the correlating OD with the Og4C3 antigen-detection ELISA ($r^2 = 0.3851$; $p < 0.005$).

Table I. — The number of dogs with either positive or negative tests related to the number of *Dirofilaria immitis* found at necropsy.

where four male and two female *D. immitis* were present with a mean microfilaraemia of $340 \pm 22/\text{mL}$, the Og4C3 ELISA, but not the VetRED™ assay, was capable of detecting circulating antigen. No false positive cases were recorded with the VetRED™ assay.

When dogs were infected with three or more *D. immitis*, 20/24 infected dogs were correctly identified using microfilarial smears and Difil only (sensitivity of 83 %); 21/24 infected dogs were identified with the VetRED™ test (sensitivity of 87 %); and 22/24 infected dogs were correctly identified with the Og4C3 ELISA (sensitivity of 92 %).

DISCUSSION

The results of this study confirm those published by Bundensen *et al.* (1990), with VetRED showing good sensitivity and specificity for detecting *D. immitis* infection, where burdens are larger than three adult worms (87 % and 100 % respectively). Comparatively, the Og4C3 ELISA performs better, with an overall sensitivity and specificity of 80 and 100 % respectively and for dogs with three worms or more, considerably higher (92 and 100 % respectively). The Og4C3 is an IgM (pentamer) monoclonal antibody, and thus has inherent restrictions in being adapted to the VetRED format which requires Fc portions of monomers which are then bound with the anti-RBC antibodies in the manufacturing of the assay ingredients. Thus, the present format of the Og4C3 antigen-detection ELISA, is unlikely to find a place in the commercial field as a quick diagnostic assay for detection of Dirofilariasis, although this does not negate its relatively good sensitivity and specificity when compared to VetRED, or its applicability in cases which require high sensitivity and specificity without time constraints on processing of samples.

If we consider the prevalence of Dirofilariasis in this survey (39 %), one can assume that in the Townsville region, a general reduction in Dirofilariasis in the order of 16 % has occurred, in comparison to previously reported surveys where prevalence rates in the order of up to 90 % have been reported (Aubrey & Copeman, 1972; Blake & Overend, 1982; Boreham & Atwell, 1988; Bundensen *et al.*, 1990; Carlisle, 1969; Carlisle & Atwell, 1985). The incidence of « occult » dogs also appears to be steadily rising, with 35 % of *D. immitis* positive cases found to be microfilariae negative in this survey. It can be concluded from this survey that successful education of residents in the Townsville region has resulted in a steady decline in the prevalence of dirofilariasis in the region, and that routine use of microfilariae detection with blood smears is becoming an increasingly inaccurate method for detection of this parasite.

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