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Evaluation of Different Methods for Diagnosis of *Dirofilaria immitis*

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Abstract: *Dirofilaria immitis*, a common parasite of the cardiovascular system of the carnivores all over the world, is reported from dogs, cats and, humans in Iran. Knott method is the most common test for its diagnosis in many regions including Iran. In the present study, during one year period from 2002 to 2003, blood samples of 110 dogs were examined using modified Knott method and commercial antigen detect test kit (WITNESS CHW II kit). All dogs were subjected to necropsy to evaluate the specificity and sensitivity of both tests. In Knott method, 22 harbored dogs (20%) were microfilaraemic of which (of the first instance), 20 dogs (18.18%) *D. immitis* and 2 (1.82%) *D. reconditum*. Accordingly, by using commercial antigen detection test kit 16 dogs (14.54%) were found to be infected with *D. immitis*. At necropsy 14 out of 110 dogs (12.73%) harbored *D. immitis* in the right ventricle of the heart. Thus, it was concluded that 6 out of 20 positive dogs with *D. immitis* were microfilaraemic and these microfilaria belonged to other filarial except *D. immitis*. To compare the findings with golden test (necropsy), the sensitivity and specificity of Knott and antigen detection test were 85.71 and 91.66%, 92.85 and 96.87%, respectively. McNemar test showed that although sensitivity of the latter was higher, differences were not significant ($p>0.05$).

Key words: *Dirofilaria immitis*, diagnosis, dog, Iran

INTRODUCTION

Canine dirofilariosis caused by *Dirofilaria immitis* is usually diagnosed by specific antigen testing and/or identification of microfilariae. However, *D. immitis* and at least six other filariae can produce canine microfilaremias with negative heartworm antigen tests (Rishniw *et al*., 2006). Accurate diagnosis of canine filariosis is essential for choosing correct therapeutic approach. Therefore, reliable methods for discriminating among the different filarial infections in dogs are needed (Casiraghi *et al*., 2006). A dot enzyme-linked immunosorbent assay (Dot-ELISA) used for detecting circulating antigens in the sera of dogs infected with *D. immitis* (Ripoll *et al*., 1991; Matsumura *et al*., 1988). Also to estimate the seroprevalence of *D. immitis* infection in domestic dogs in Taiwan, they utilized a commercial ELISA kit (Snap, IDEXX, USA) for detecting circulating antigens released by female adult worms (Fan *et al*., 2001). In the present study, the attempts were made to compare modified Knott method with that of antigen detection test in order to present more accurate method and to determine the prevalence of the infection in stray dogs of Golestan province, north of Iran.

MATERIALS AND METHODS

Blood samples: In a period of one year, from 2003-2004, 110 blood samples taken from saphen or cephalic vein of stray dogs in Golestan province (north of Iran) were examined using modified Knott method and an antigen detection test kit (WITNESS CHW II kit) and then necropsy was carried out to confirm the accuracy of these methods.

Modified Knott method: In Knott method, the sediments were searched for microfilariae and identification of which was performed using the key presented by Etinger and Feldmen (2000) key.

Heartworm antigen test kit (WITNESS CHW II kit): In antigen detection test, examination procedures were followed according to manufacturer's recommendation. The appearance of pink or purple lines in window 2 and 3, respectively were taken as positive with *D. immitis* (Fig. 1).

Necropsy: All dogs were subjected to necropsy and then pulmonary arteries and hearts were searched for the

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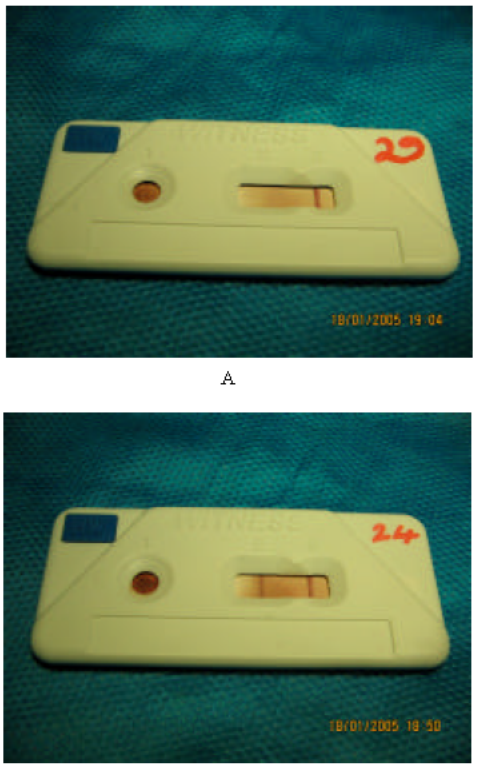

A

B

Fig. 1: Results of WITNESS CHW II kit. A: Negative result (The presence of line in win. 3 and its absence in win. 2). B: Positive result (The presence of both lines in win. 2 and 3)

presence of adult *D. immitis*. The collected worms were counted in preserved in 70% alcohol containing 5% glycerin.

Statistics: McNemar test has been used for evaluation of our findings.

RESULTS

The results of both tests are summarized in Table 1. Necropsy as a golden test showed that 14 out of 110 examined dogs (12.73%) were found to be infected with *D. immitis* whereas in Knott method 20 dogs (18.18%) and by antigen detection test 16 dogs (14.54%) showed microfilaria of *D. immitis* in that order. Thus it can be postulated that in Knott method, 6 dogs were microfilaraemic and these microfilaria were belong to other filarial except *D. immitis* (5.45% false positive results) and two out of 90 non infected dogs shown by Knott method (1.82%) were positive at necropsy (false negative = occult infection). Meanwhile in antigen detection test 2 out of 16 dogs infected with heartworm antigen (1.82%) were not positive at necropsy (false positive) and 1 (0.91%) out of non infected dog was positive at necropsy (false negative = occult infection). Although sensitivity of antigen detection test was higher, no difference was shown using McNemar test ($p>0.05$). At necropsy, 14 out of 110 dogs were found to be infected with *D. immitis*. The number of worms found ranged from 1-7 of which two dogs harbored only one worm in the right ventricle that proves two cases of occult infection.

DISCUSSION

The results of this investigation show that in Knott method and antigen detection test kit, the sensitivity and specificity were high (85.71 and 91.66%, 92.85 and 96.87%), respectively. Present findings in the case of antigen detection test were similar with many other workers using different commercial kits (Atkins, 2003; Courtney and Zeng, 2001). This shows that antigen detection test regardless of the type of kit is a reliable method and its accuracy is not microfilaraemia dependent, thus can be used to detect amicrofilaraemic or occult infection or for rapid screening of heartworm infection. Knott method was also accurate enough for diagnosis of a high percentage of infected dogs with *D. immitis* in the present study. Some workers believe that this is not as good as antigen detection test (Atkins, 2003), although their advantages make of the latter a choice method. It seems likely that occult heartworm due to treatment of infection dogs with Ivermectine or Levamisole is the main source of high occult infection in many country (Rawlings *et al*., 1982), thus low occult infection reported in this survey seems to be reasonable because anti *D. immitis* treatment is not practiced at all in Iran, except in experimental study (Eslami *et al*., 2004); although according to clinical signs and positive response to treatment with Melarsomine dichlorhydrate 3 out of 30 infected (10%) harbored occult infection, a finding similar to present investigation. Accordingly, 18.18 and

Table 1: Results of modified Knott method and commercial heartworm antigen test (WITNESS CHW II kit) from 110 dogs and comparison of them

	No. of samples								
Test	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	Accuracy (%)	Predictive value (+)	Predictive value (-)
Modified Knott method	12	88	8	2	85.71	91.66	90.90	60.00	97.77
WITNESS CHW II kit	13	93	3	1	92.85	96.87	96.36	81.25	98.93

1.82% of 110 dogs of Golestan province harbored *D. immitis* and *D. reconditum*, respectively. These are in line with the findings of Meshgi *et al.* (2002) for sheepdogs of Tabriz, North West of Iran for heartworm infection (18.68%) and *D. reconditum* (4.94%), respectively; although it is much higher previous rate (4%) reported for *D. immitis* (Sadighian, 1969) in the same geographical region. In sum, it can be concluded that diagnostic value of Knott method is similar to antigen detection test for cases in which occult infection is not a serious problem.

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REFERENCES

Atkins, C.A., 2003. Comparison of results of three commercial heartworm Antigen test kits in dogs with low heartworm burdens. JAVMA., 222: 1221-1223.

Casiraghi, M., C. Bazzocchi, M. Mortarino, E. Ottina and C. Genchi, 2006. A simple molecular method for discriminating common filarial nematodes of dogs (*Canis familiaris*). Vet. Parasitol., 141: 367-372.

Courtney, C.H. and Q.Y. Zeng, 2001. Comparison of heartworm antigen test kit Performance in dogs having low heartworm burdens. Vet. Parasitol., 96: 317-322.

Eslami, A., J. Ashrafi Helan and B. Meshgi, 2004. Canine heartworm, clinical Presentation and treatment. Ind. Vet. J., 21: 201-205.

Etinger, S. and F.C. Feldman, 2000. Textbook of Small Animal Internal Medicine. W.B. Saunder, New York.

Fan, C.K., K.E. Su, Y.H. Lin, C.W. Liao, W.Y. Du and H.Y. Chiou, 2001. Seroepidemiologic survey of *Dirofilaria immitis* infection among domestic dogs in Taipei City and mountain aboriginal districts in Taiwan (1998-1999). Vet. Parasitol., 102: 113-120.

Matsumura, K., S. Wakatsuki, R. Endo, K. Tanaka, T. Inoue and H. Matsuda, 1988. A rapid detection of circulating antiges of *Dirofilaria immitis* in dogs by a dot enzyme-linked immunosorbent assay. FEMS Microbiol. Immunol., 1: 145-149.

Meshgi, B., A. Eslami and J. Ashrafi Helan, 2002. Epidemiological survey of blood filariae in rural and urban dogs of Tabriz. J. Fac. Vet. Med. Tehran. Univ., 57: 59-63.

Rawlings, C.A., D.L. Dawe, J.W. McCall, J.C. Keith and A.K. Prestwood, 1982. Four types of occult *Dirofilaria immitis* infection in dogs. JAVMA., 180: 1323-1326.

Ripoll, B.D., A.M.E. Hernandez, M.C.M. Valonga and C.F. Villalvilla, 1991. Excretion-secretion antigens from adult *Dirofilaria immitis* in the diagnosis of Human filariasis by solid phase immunoenzyme assay. Rev. Cubana. Med. Trop., 43: 162-166.

Rishniw, M., S.C. Barr, K.W. Simpson, M.F. Frongillo, M. Franz and J.L. Dominguez Alpizar, 2006. Discrimination between six species of canine microfilariae by a single polymerase chain reaction. Vet. Parasitol., 135: 303-314.

Sadighian, A., 1969. Helminth parasites of stray dogs and jackals in Shahsavar area, Caspian region, Iran J. Helminth, 2: 372-374.