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SHORT COMMUNICATION

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Evaluation of quantitative buffy coat analysis in the detection of canine *Dirofilaria immitis* infection: a model to determine its effectiveness in the diagnosis of human filariasis

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Abstract Quantitative buffy coat (QBC) analysis has been reported to have a high degree of methodical sensitivity in the detection of human filariasis. This study was conducted to evaluate its usefulness in the diagnosis of filariasis using a *Dirofilaria immitis*/dog model. By necropsy of 244 stray dogs, 40.6% of the animals were found to harbor 1–58 worms of *D. immitis* (mean 6.5 ± 8.4 worms/infected dog). The QBC analysis and thick blood smear (TBS) method detected microfilaremia in 31.6% and 21.3% of these dogs, respectively. The results of these two methods were highly correlated with the presence of bisexual worms in the dogs. The QBC analysis was more sensitive (55% versus 39%) and efficient (79% versus 72%) than the conventional TBS method. However, accurate speciation of the microfilariae was impossible using the QBC analysis. Although this technique is more sensitive, simpler, and less time-consuming and does not require as much skill or experience in comparison with the conventional TBS method, the failure in speciation of the parasites may limit its usefulness.

Quantitative buffy coat (QBC) analysis is a relatively new technique in the diagnosis of blood parasites. It has been reported to exhibit 100% methodical and diagnostic sensitivity as compared with the conventional thick blood smear (TBS) method in the diagnosis of bancroftian filariasis (Long et al. 1990). Moreover, it enables identification of the microfilariae (mf) up to the species level (Freedman and Berry 1992; Bawden et al. 1994). However, no information is available on the evaluation of this technique based on autopsy. Since it is not easy to compare the results of QBC analysis with autopsy data, the *Dirofilaria immitis*/dog model is an excellent model for evaluation of this technique. In this study we evaluated the efficiency of QBC analysis by comparing the results of QBC analysis and the TBS method with the necropsy findings obtained among stray dogs in Taiwan.

A total of 244 stray dogs were obtained from the Municipal Institute for Animal Health. These dogs were captured in Taipei City by the environmental protection units and were determined to be over 1 year of age by the presence of adult canine teeth.

The dogs were euthanized between 9 a.m. and 12 noon by intramuscular injection of ketamine hydrochloride followed by intracardiac injection of sodium thiopentone 15 min later. Immediately after euthanasia, whole-blood samples were collected from the axillary artery into tubes coated with ethylenediaminetetraacetic acid (EDTA). After removal of the heart and lungs from the carcass, the venae cavae, right atrium, right ventricle, and all major branches of the pulmonary arteries were opened and examined thoroughly for heartworms. The number, sex, and developmental stage of heartworms found in each dog were determined. Adolescent and adult worms were distinguished according to the criteria of Orihel (1961).

Each specimen (60 μl) was examined using a commercially available QBC assay kit (Becton-Dickinson Company, Franklin Lakes, N.J., USA). The procedure was carried out according to the instructions of the manufacturer and the centrifuged capillary tube was examined under a compound microscope equipped with the ParaLens adapter. From the same specimen, Giemsa-stained TBSs were prepared with the same volume of blood according to the method described by Ash and Orihel (1987) and were examined under a compound microscope for mf. The speciation of the mf was confirmed using a modified Knott's test (Newton and Wright 1956). Dogs with other canine filarial worms but without *D. immitis* mf were excluded from the evaluation.

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The chi-square test was used to compare rates. Spearman's rank correlation was performed to evaluate the relationship between necropsy findings and results of the mf-detecting methods. The sensitivity and efficiency of the two methods were also calculated (Kirkwood 1988).

Of the 244 strays dogs necropsied, 99 (40.6%) were infected with 7–58 worms of *D. immitis*. The average worm burden for the infected dogs was 6.5 ± 8.4 worms. Among the infected dogs, 7.1% harbored only adolescent worms; 17.2%, only male adult worms; 10.1%, only female adult worms; and 5.1%, single-sex adult worms and adolescents. Adult worms of both sexes were found in 60.6% of the infected dogs (Table 1).

By the QBC analysis, 77 (31.6%) dogs were found to be microfilaremic, whereas the TBS method demonstrated only 52 (21.3%) dogs with microfilaremia. However, 28 microfilaremic dogs without worms in their cardiopulmonary system were found to be infected only with *Dipetalonema reconditum* using the modified Knott's test. These dogs were excluded from the evaluation. The results obtained by these two mf-detecting methods were highly correlated with the presence of worms of both sexes (QBC analysis: $r = 0.81$, $P < 0.001$; TBS method: $r = 0.73$, $P < 0.001$), although their correlations with the total number of worms were also statistically significant (QBC analysis: $r = 0.55$, $P < 0.001$; TBS method: $r = 0.49$, $P < 0.001$). Moreover, the QBC analysis was more sensitive and efficient than the TBS method (Table 2).

Species identification of human filariasis had been made by observation of centrifuged parasites stained with acridine orange under a conventional compound microscope equipped with the ParaLens adapter (Long et al. 1990; Bawden et al. 1994). However, we could not accomplish accurate speciation using the QBC analysis. The modified Knott's test (Newton and Wright 1956) was employed to determine the species of mf. This test offers more advantages in the assessment of mf morphology than other lysis concentration techniques (Atwell 1988). Although the QBC analysis failed to determine the species of mf, it was more sensitive and efficient than the conventional TBS method. These results confirmed those reported by Long et al. (1990).

Table 2 Comparison of the effectiveness of the QBC and TBS techniques in the diagnosis of canine *D. immitis* infection among 216 dogs

	QBC	TBS
Positive test results for infected dogs (*n*)	54	39
Negative test results for infected dogs (*n*)	45	60
Positive test results for uninfected dogs (*n*)	0	0
Negative test results for uninfected dogs (*n*)	117	117
Sensitivity (%)	55	39
Efficiency (%)	79	72

Since acridine-stained infective stages can always be demonstrated under the microscope, the QBC analysis is useful in the diagnosis of malaria and trypanosomiasis (Spielman et al. 1988; Anthony et al. 1992; Bailey and Smith 1992). However, this method is capable of detecting only patent filarial infections. For dog heartworm infection, amicrofilaremia may occur in precardial, prepatent, unisexual, occult, postpatent, and post-treatment sterile infections (Wong 1987). Moreover, parasitology methods can detect only up to 70% of infected dogs (Sisson et al. 1985). Although assays using monoclonal antibodies for the detection of circulating antigens can detect amicrofilaremic infected dogs, these assays rely on the presence of fecund female worms (approximately 6.5 months postinfection; American Heartworm Society 1992) and are incapable of diagnosing prepatent infections. In addition, false-negative findings are likely to occur in dogs with low worm burdens (Brunner et al. 1988; Bundesen et al. 1990). The diagnosis of human filarial infections may involve similar problems.

In this study, QBC analysis and the TBS method detected 54.5% and 39.4% of canine heartworm infections, respectively. The results were also demonstrated to be correlated with the number of worms and strongly correlated with the presence of adult worms of both sexes. Fortunately, dogs harboring adult worms of both sexes were in the majority, and the QBC analysis revealed 81.7% of the infected dogs. Although QBC analysis cannot reveal the true infection status, the results of the present study signify its diagnostic sensitivity. In addition, this method is simpler and less

Table 1 Necropsy findings of *Dirofilaria immitis* and microfilaremia detected by the QBC and TBS techniques in 244 stray dogs from North Taiwan

Infection status	Necropsy findings of *D. immitis*		Number with microfilaremia as determined by	
	Dogs (*n*)	Worms/dog (*n*)	QBC	TBS
Negative	145	0	23	13
Only adolescent worms	7	1–8	0	0
Only male adult worms	17	1–2	4	1
Only female adult worms	10	1–4	0	0
Single-sex adult worms and adolescents	5	2–6	1	0
Only adult worms of both sexes	53	2–58	44	32
Adult worms of both sexes and adolescents	7	5–31	5	6
Totals	244	0–58	77	52

time-consuming and does not require much skill or experience. However, the failure in identifying the species of the parasites may limit its usefulness under field or clinical conditions.

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