NOTE *Parasitology*

Comparison of Microfilaria Concentration Method for *Setaria digitata* Infection in Cattle and for *Dirofilaria immitis* Infection in Dogs

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ABSTRACT. Several peripheral blood microfilaria concentration methods that use Acetone (Acetone test), 2% formalin (modified Knott method), 5% Tween 20 solution, distilled water, 1% or 0.1% SDS were compared for their efficacy in detecting *Setaria digitata* microfilaria in cattle. The Acetone test was found to be more efficacious than the modified Knott method or the 5% Tween 20 solution test for detecting the *S. digitata* microfilaria in bovine blood. However, besides the Acetone test, the modified Knott method was also found to be suitable for *Dirofilaria immitis* microfilaria detection in dogs. SDS and distilled water were found not to be effective as hemolytic agent for the disruption of the red blood cell of both the cattle and dogs. Thus, the Acetone test is recommended for the prima ry screening of microfilaremia of *S. digitata* in cattle.

KEY WORDS: concentration method, *Dirofilaria immitis*, *Setaria digitata*.

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Setaria digitata is a common filarial nematode that parasitize in the peritoneal cavities of cattle and buffalo [8], as well as causing cerebrospinal setariosis in goat [4] and cattle [7] in Taiwan. For the primarily screening of this filarial infection in cattle, the direct blood smear method is commonly used. The modified Knott method has been widely used in the diagnosis of human Bancroftian filariasis [6, 9] and canine dirofilariasis [2, 3, 5]. When this microfilaria (mf) concentration method was applied for the detection of *S. digitata* microfilaremia in cattle, problems that were not seen when this method was used in dogs for *Dirofilaria immitis* detection, such as incomplete hemolysis of the bovine erythrocytes, were observed. Besides the direct smear method, comparative study on the use of the most optimum microfilaria concentration technique for *S. digitata* in cattle had not been reported. In this study, we compared the efficiency of Acetone (Acetone test), 2% formalin (modified Knott test), Tween 20 solution, distilled water, 1% SDS and 0.1% SDS as hemolytic solution for disrupting the blood cells in the microfilaria concentration method.

Eleven bovine blood samples from Taichung county, Taiwan, were collected by venipuncture of the caudal vein using EDTA-containing syringe. Cattle used in this study have been suspected to be infected with *S. digitata*. Blood of dogs that had been confirmed to be infected with *D. immitis* were also collected as control. The dogs were randomly selected from animal shelters in Taichung county, Taiwan.

For the Acetone mf concentration technique, one m*l* of the whole blood was placed into a centrifuge tube containing 9 m*l* of acetone hemolytic solution (5 m*l* acetone, 5 m*l* 0.5% methylene blue aqueous solution added to 90 m*l* of distilled water), and mixed thoroughly. After centrifuging at 160 × g for 5 min, the supernatant was discarded, and 9 m*l* distilled water added to the 1 m*l* of the residual fluid containing the microfilaria. The test-tubes were centrifuged again at 160 × g for 5 min. After decanting the supernatant, the sediments were spread onto a slide glass, covered with a cover slip of 24 × 32 mm, and examined under light microscope.

In the modified Knott's mf concentration technique, one m*l* of whole blood was added into a test-tube containing 9 m*l* of 2% formalin. After mixing thoroughly, the blood sample was centrifuged at 160 × g for 5 min. The supernatant was discarded, and 9 m*l* of 0.1% methylene blue solution added to the 1 m*l* of residual fluid containing the microfilaria. The test-tube was again centrifuged at 160× g for 5 min. The sediment was placed onto a glass slide, covered with a cover slip, and then examined under light microscope.

For the distilled water, 5% and 0.5% Tween 20, 1% and 0.1% SDS mf concentration techniques, the aforementioned liquids were individually used as hemolytic solution instead of the 2% formalin solution in the modified Knott's mf concentration technique. After the first centrifugation, 9 m*l* of 0.1% methylene blue solution were added to the 1 m*l* of residual fluid containing the microfilaria. For the second centrifugation and the remaining procedure, the process was repeated as described above.

For the microfilaria density determination by direct smear count, a total of 200 μl whole blood for each cattle or dog were examined. This was done by placing 20 μl of whole blood onto the slide glass, covering it with a cover slip, and counting all the microfilaria on the slide glass. Counting was conducted 10 times at 20 μl each for each of the blood sample.

For statistical analysis, randomized complete block design was used. Data of microfilaria count obtained from 20 μl blood samples of either cattle or dogs were entered

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Table 1. Comparison of various microfilaria concentration tests for *Setaria digitata* microfilaremia in cattle

Cattle no.	Direct smear[a)]	Mf concentration method			
		Acetone	Knott	Tween 20 (5%)	D.W.
1	5.6 ± 1.52 [C]*	13.0 ± 3.0 [A]	4.8 ± 2.17 [CD]	10.0 ± 2.12 [B]	2.4 ± 1.52 [D]
2	4.6 ± 3.21 [B]	51.2 ± 17.12 [A]	5.4 ± 2.61 [B]	2.8 ± 1.64 [B]	8.4 ± 3.44 [B]
3	3.0 ± 1.52 [B]	38.6 ± 36.20 [A]	7.6 ± 3.13 [B]	2.6 ± 2.05 [B]	0.8 ± 0.89 [B]
4	1.0 ± 0.55 [BC]	4.7 ± 2.39 [A]	2.3 ± 1.30 [B]	0 [C]	1.7 ± 2.61 [BC]
5	0.4 ± 0.89 [B]	2.2 ± 1.92 [A]	1.1 ± 1.64 [AB]	nd	0.5 ± 0.71 [B]
6	1.0 ± 0.71 [BC]	3.2 ± 2.77 [AB]	4.0 ± 1.58 [A]	3.6 ± 1.14 [A]	nd
7	0.6 ± 0.89 [A]	0.6 ± 0.89 [A]	1.0 ± 1.22 [A]	0.4 ± 0.55 [A]	nd
8	0.4 ± 0.55 [A]	0.4 ± 0.55 [A]	0.2 ± 0.45 [A]	0 [A]	nd
9	0.2 ± 0.45 [C]	3.0 ± 2.74 [AB]	3.2 ± 0.84 [A]	1.0 ± 1.0 [BC]	nd
10	0.2 ± 0.45 [A]	0.8 ± 0.45 [A]	0.6 ± 0.55 [A]	0.4 ± 0.89 [A]	nd
11	0 [B]	0.6 ± 0.55 [A]	0.2 ± 0.45 [AB]	0 [B]	nd

a) For each cattle, ten 20 μl blood sample were prepared, and all the mf present counted. Values are means ± S.D.
nd: Not done.
*: Analysis using Duncan's multiple range test in completely randomized design. Different alphabet letters along the same horizontal lane denotes presence of significant difference among the various concentration methods.

Table 2. Comparison of various concentration tests for *Dirofilaria immitis* microfilaremia in dogs

Dogs no.	Direct smear[a)]	Mf concentration method		
		Acetone	Knott	Tween 20 (5%)
1	805.6 ± 171.07 [A]*	868.8 ± 287.9 [A]	970.2 ± 224.92 [A]	nd
2	80.8 ± 19.23 [B]	103.4 ± 15.26 [B]	170.8 ± 20.02 [A]	nd
3	1.2 ± 1.64 [A]	2.6 ± 1.14 [A]	1.6 ± 0.55 [A]	nd
4	40.6 ± 25.76 [B]	29.4 ± 8.79 [B]	68.6 ± 18.77 [A]	32.2 ± 7.29 [B]
5	9.2 ± 6.06 [B]	24.6 ± 8.23 [A]	17.2 ± 4.44 [AB]	11.0 ± 5.61 [B]
6	0 [A]	0.2 ± 0.45 [A]	0.2 ± 0.45 [A]	0.2 ± 0.45 [A]
7	1.6 ± 1.34 [A]	3.2 ± 2.39 [A]	2.4 ± 1.82 [A]	1.8 ± 1.30 [A]

a) For each dog, ten 20 μl blood sample were prepared, and all the mf present counted. Values are means ± S.D.
nd: Not done.
*: Analysis using Duncan's multiple range test in completely randomized design. Different alphabet letters along the same horizontal lane denotes presence of significant difference among the various concentration methods.

into a computer file (SAS: Statistical Analysis System) and analyzed using programs available in SAS. Average microfilaria counts were compared. Duncan's multiple range test was used to identify which group differed significantly from the other.

The results of the various mf concentration tests for *S. digitata* microfilaremia in cattle are shown in Table 1. For comparison, the results of various microfilaria concentration tests for *D. immitis* microfilaremia in dogs are shown in Table 2. The resulting microscopic picture of the *S. digitata* microfilaria from cattle obtained by the Acetone concentration test is shown in Fig. 1 and that by the modified Knott method in Fig. 2. Much debris could be seen accumulating around the microfilaria in the latter method. However, the modified Knott method was found to be suitable for the detection of *D. immitis* microfilaria in the blood sample of dogs. We also found that the mf of both *S. digitata* and *D. immitis* were covered with much debris when different concentration of SDS (1%, 0.1%) and distilled water were used as hemolytic agents.

In some samples in our study, although no microfilaria was found by the direct smear method, at least one microfilaria per 100 μl of blood was found after treatment with the mf concentration methods such as the Acetone test, modified Knott method and 5% Tween 20 test. These low-level microfilaremia had been reported to give negative or very weak reaction in the antigen test such as DiroCHEK for *D. immitis* [1].

In this study, the Acetone concentration test was found to be useful for the screening of *S. digitata* microfilaria in cattle instead of the direct smear method. Although the Knott test is useful as a diagnostic method for microfilaremia of dogs, it is not recommended for detecting microfilaria in blood sample of cattle because the resulting precipitate made identification of the microfilaria quite difficult. Probably, the component of cattle blood might be different from those of the carnivorous animals because hemolysis occurred more easily in the latter blood. However, further study to elucidate the differences between cattle and dog blood components will be needed. Therefore, we recommended that the Acetone microfilaria concentration test be used for the detection *S. digitata* microfilaria as a primary screening method, but the Knott test can still be applied for canine dirofilariasis.

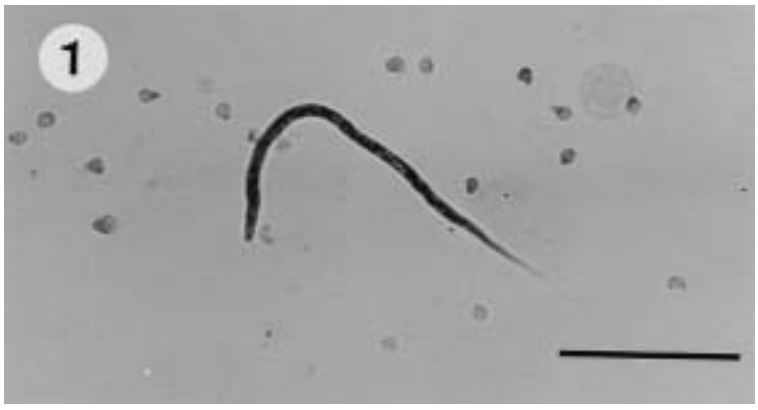

Fig.1. *Setaria digitata* microfilaria detected by the Acetone method. Bar=100 μm.

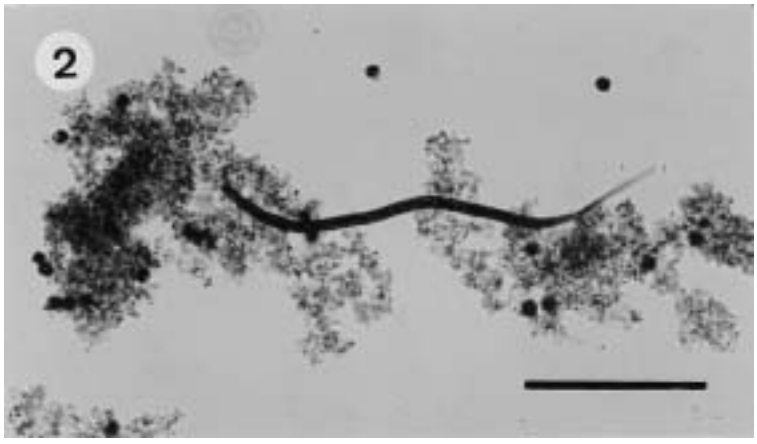

Fig. 2. *Setaria digitata* microfilaria detected by the modified Knott method. Bar=100 μm.

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