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Use of Kidney Impressions for the Detection of Trypanosomes of Anura

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ABSTRACT: The sensitivities of three techniques used for detecting infections of *Trypanosoma* spp. in frogs (*Rana* spp.) were compared. In total, 52 of 99 frogs had detectable infections of *T. rotatorium*, *T. chattoni*, *T. pipientis* or *T. ranarum*. Two or more *Trypanosoma* spp. were detected in 12 frogs. Microscopic examination of stained kidney impressions (KIT) was more sensitive than either hematocrit centrifugation (HCT) or wet-mount examination (WME) in detecting *T. rotatorium* and *T. chattoni*. The HCT was more sensitive in detecting *T. pipientis* and *T. ranarum*. Four infections of *T. rotatorium* that were missed using the HCT were detected using the WME; one of these was missed using the KIT. Success of the KIT may be related to size of the trypanosome while success of the HCT may be related to size, motility or specific gravity of the trypanosome.

Key words: *Trypanosoma* spp., frogs, *Rana* spp., detection, stained kidney impression technique, hematocrit centrifugation technique, wet-mount examination, comparative study.

Microscopic examination of stained blood films was commonly used to detect infections of *Trypanosoma* spp. in many species of Anura (*Rana* spp.) (Werner and Walewski, 1976; Barta and Desser, 1984). The hematocrit centrifugation technique (HCT), however, was more sensitive than examination of blood films (Woo, 1983). Despite this, not all infections of trypanosomes in leopard frogs, *Rana pipiens*, were detected by Woo (1983) using the HCT. In addition, early workers observed *Trypanosoma rotatorium* and *T. chattoni* more readily in renal blood vessels and in stained kidney impressions than in peripheral blood of frogs (Kudo, 1922; Fantham et al., 1942). The present study was undertaken to compare the sensitivity of the examination of stained kidney impressions with that of other techniques in detecting infections of trypanosomes in frogs.

Ninety nine frogs (41 leopard frogs; 46 mink frogs, *R. septentrionalis*; eight green frogs, *R. clamitans*; two bullfrogs, *R. catesbeiana*; and two wood frogs, *R. sylvatica*) were collected from Long Point (42°35′N, 80°27′W), Guelph (43°32′N, 80°13′W) and Orangeville (43°56′N, 80°05′W) (Ontario, Canada). Frogs were killed within 2 days of capture by immersion in tricaine methane sulphonate (Syndel Laboratories Ltd., Vancouver, British Columbia, Canada V6P 4J6). The heart and kidneys were exposed through a ventral incision and blood was collected into at least four heparinized capillary tubes.

For wet mount examination (WME) a drop of fresh blood was dispensed onto a microscope slide and covered with a glass coverslip (18 × 18 mm). The number of organisms in 50 microscope fields (objective 10×, ocular 10×) were counted.

For the hematocrit centrifugation technique three heparinized capillary tubes of fresh blood were centrifuged at 13,000 *g* for 4 min. Following centrifugation, the tubes were examined using an inverted microscope (objective 10×, ocular 10×) (Woo, 1969a). When parasites were observed, a smear was made from the buffy layer of each tube. Smears were air dried and fixed in 100% ethanol then in buffered 10% formalin (pH 7.2). These were then air dried and stained with Giemsa's stain for 40 min.

For the kidney impression technique (KIT) a kidney was removed from each frog and cut along its long axis. At least 15 impressions were made from the cut surface onto a microscope slide. Kidney impressions from each frog were fixed and stained with the same procedure used for smears of buffy layer and examined using a compound microscope (objective 100×, ocular 10×) for exactly 15 min.

TABLE 1. Prevalence of infections with various *Trypanosoma* spp. in different anuran hosts.

Trypanosoma spp.	*Rana* spp.[a]				
	1	2	3	4	5
pipientis	20/46[b] (44)	19/41 (46)	1/8 (13)	0/2 (0)	0/2 (0)
rotatorium	0/46 (0)	9/41 (22)	5/8 (63)	0/2 (0)	0/2 (0)
chattoni	0/46 (0)	8/41 (20)	0/8 (0)	0/2 (0)	0/2 (0)
ranarum	1/46 (2.2)	4/41 (10)	0/8 (0)	0/2 (0)	0/2 (0)

[a] 1, *R. septentrionalis*; 2, *R. pipiens*; 3, *R. clamitans*; 4, *R. catesbeiana*; 5, *R. sylvatica*.
[b] Number infected/number examined (percent).

TABLE 2. Detection of infections of *Trypanosoma* spp. using various techniques.

Trypanosoma spp.	Technique[a]			Total number of infections
	WME	HCT	KIT	
T. rotatorium	8[b]	7	13	14
T. chattoni	0	1	8	8
T. pipientis	13	40	5	40
T. ranarum	3	5	2	5

[a] WME, wet mount examination; HCT, hematocrit centrifugation technique; KIT, kidney impression technique.
[b] Detected four infections of *T. rotatorium* that were missed by HCT, one of these was also missed by KIT.

Specific diagnoses of *Trypanosoma* spp. are those of Woo (1969b) and Barta and Desser (1984); 52 of 99 frogs had detectable infections of trypanosomes. Of these, 12 were infected with more than one species of *Trypanosoma*. Prevalence of each *Trypanosoma* sp. in all hosts is given in Table 1. The KIT detected 13 of 14 infections of *T. rotatorium* (eight of nine in *R. pipiens*, five of five in *R. clamitans*) and eight of eight infections of *T. chattoni* in *R. pipiens*. However, the KIT detected only five of 40 infections of *T. pipientis* in *R. pipiens* and two of five infections of *T. ranarum* (one in *R. pipiens*, one in *R. septentrionalis*). Conversely, the HCT detected all infections of *T. pipientis* and all infections of *T. ranarum*. However, the HCT detected only seven of 14 infections of *T. rotatorium* (five of nine in *R. pipiens*, two of five in *R. clamitans*) and only one of eight infections of *T. chattoni* (see Table 2). The WME detected four infections of *T. rotatorium* that were missed by the HCT and one of these was also missed by the KIT.

These results demonstrate clearly the usefulness of the KIT for the detection of infections of *T. rotatorium* and *T. chattoni* but not of *T. pipientis*. This confirms an earlier study in which *T. pipientis* was detected more readily by using the HCT (Woo, 1983). Occurrence of *T. ranarum* in one of 46 *R. septentrionalis* is a new host record. The parasite was detected in this host by using both the KIT and the HCT.

In this study, *T. rotatorium* and *T. chattoni* were observed more readily in kidney impressions than in peripheral blood, confirming an earlier report (Fantham et al., 1942). Kidney contains extensive capillary plexi and may differentially entrap trypanosomes according to size. For example, the mean body width of *T. rotatorium* and *T. chattoni* is 20.0 and 37.1 μm, respectively, while that of *T. pipientis* is only 3.0 μm (Woo, 1969b; Jones and Woo, 1986). Therefore, success of the KIT in detecting infections of trypanosomes may be directly related to size of the parasite.

The sensitivity of the HCT may be related to specific gravity, size or motility of trypanosomes (Walker, 1972; Woo and Rogers, 1974). Thus, *T. rotatorium* and possibly *T. chattoni* sedimented with blood cells and this evidently prevented their being detected using the HCT. This was supported by the WME detecting four infections of *T. rotatorium* that were missed by the HCT. In this study, five of five infections of *T. ranarum* were detected using the HCT. Woo (1983) detected only 10 of 13 infections of *T. ranarum* using the same technique. The occurrence of two distinct morphological types of *T. ranarum* (Woo, 1969b) may explain this difference.

Previous studies (Woo, 1969a; Woo and

Bogart, 1984) have concluded that the HCT, because of its speed and sensitivity, should be the technique of choice in detecting infections of all trypanosomes in frogs. The results of this study demonstrate that the KIT is a more sensitive technique than the HCT in detecting larger frog trypanosomes such as *T. rotatorium* and *T. chattoni*. It is suggested that both the KIT and the HCT should be used in future surveys to obtain accurate prevalence data for all species of trypanosomes in frogs.

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