STUDIES ON FROG TRYPANOSOMIASIS
II. SEASONAL VARIATIONS IN THE PARASITEMIA LEVELS OF *TRYPANOSOMA ROTATORIUM* IN *RANA CLAMITANS* FROM LOUISIANA

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ABSTRACT

Seasonal variations in the parasitemia level of *Trypanosoma rotatorium* infections of *Rana clamitans* are described. The seasonal cycle of parasitemia is not influenced by the sex of the frog. Preliminary data also suggest that in frogs over 5.1 cm long, the age of the frog had no apparent relation to the percentage of infected frogs or the parasitemia level. Various factors, i.e., water temperature, mating cycle, changes in photoperiod, which might influence the seasonal cycle are discussed. The importance of these results in epidemiological studies of parasitic infections is also discussed.

INTRODUCTION

Numerous species of trypanosomes have been described from various species of frogs in the United States. Much of the early taxonomic work has been reviewed by Diamond (1965). He also discussed the biology of trypanosomes infecting frogs, including methods of cultivation, vectors involved in transmission, laboratory maintenance of frogs, vectors, and so forth.

Nigrelli (1945) described a morphological type of trypanosome infecting *Rana clamitans*. He identified this species as *Trypanosoma rotatorium* (Mayer, 1843). This species is the one reported in this paper. Diamond (1965) believes that *T. rotatorium* (Mayer, 1843) has not been described from frogs captured in America. He did not, however, name the species which has been regarded as *T. rotatorium* by Nigrelli (1945). Until more concrete evidence regarding the true identity of *T. rotatorium* (Mayer, 1843) of Nigrelli (1945) becomes available, we will continue to identify this trypanosome as *T. rotatorium*.

There have been few or no reports on the incidence or intensity of *T. rotatorium* infections of *R. clamitans*. The work to be reported here describes the parasitemia levels of *T. rotatorium* infections of *R. clamitans* captured near Norco, Louisiana throughout a 1½ year period.

MATERIALS AND METHODS

The frog, *Rana clamitans*, was collected throughout the year from swamps in the Goodhope oil field near Norco, Louisiana. It was maintained in glass aquaria and exposed to the normal daily fluctuations of light. Subsequent to its capture, each frog was weighed, measured from the tip of snout to vent, and if possible its sex determined from external characters. To determine parasitemia levels, a vein in the upper corner of the jaw was punctured, blood extracted with a micro Pasteur pipette, and placed on a microslide. Slides were examined under low power (125 ×) with the light microscope. Frogs were examined within 3 days of capture, almost always between the hours of 12:00 noon and 7:00 p.m. This is the period of maximum parasitemia level (Southworth, et al., 1968). Trypanosomes contained in a drop of blood covered with a 22 mm square cover slip were counted and averaged for a minimum of 30 low power fields. Only those fields which contained a relatively constant number of red blood cells were examined. In addition, a drop of blood under a 22-mm-square cover slip was systematically

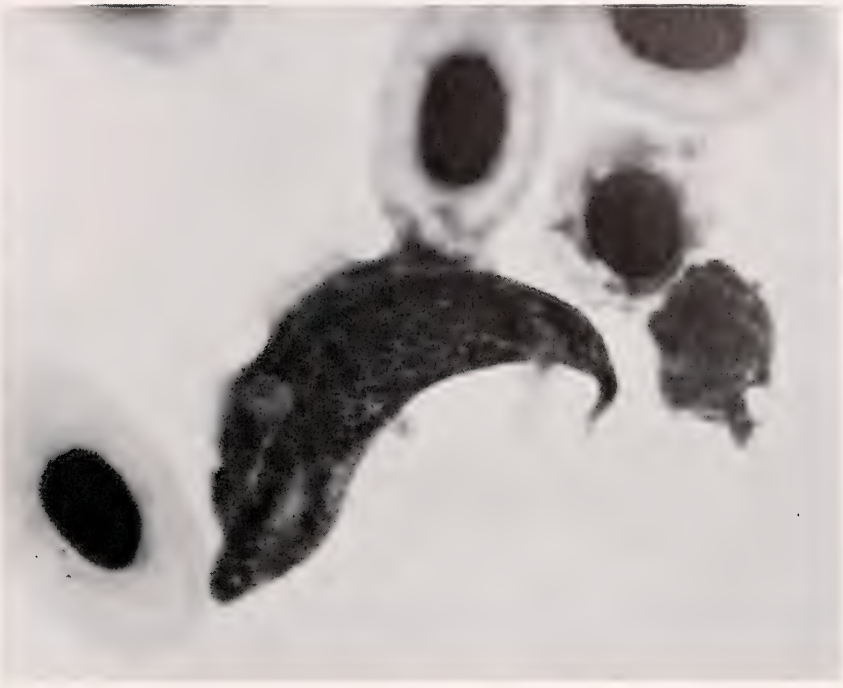

Figure 1. *Trypanosoma rotatorium* in *Rana clamitans* from Louisiana (1800×). The measurements made on trypanosomes from stained blood smears are as follows: Posterior end to kinetoplast, 4.92 μ (9); Posterior end to center of nucleus, 16.5 μ (38); Posterior end to anterior end, 46.2 μ (49); with including undulating membrane, 14.5 μ (49); No free flagellum. The numbers in parentheses are the number of measurements made.

examined from those frogs which appeared negative for trypanosomes. In one collection (30 Jan. 1967), the frogs were examined for *T. rotatorium* every 4 hours over a 24-hour period.

Trypanosoma rotatorium was differentiated from the other morphological types of trypanosomes found in the fresh blood preparations of *R. clamitans* by their characteristic size, shape, absence of a free flagellum, and type of movement. *Trypanosoma rotatorium* was the largest trypanosome observed, generally assumes a U-shape, and moves with closed end of the U leading.

For the measurement of trypanosomes, blood smears were prepared and stained with Giesma's stain. Measurements were made under oil immersion with the light microscope (1250×).

Results

In examining the blood of 486 *Rana clamitans* collected over a period of 1½ years 81 per cent were found to be infected with *Trypanosoma rotatorium* (Mayer, 1843). The measurement and morphology of this trypanosome corresponds exactly to the elongate forms of *T. rotatorium* described by Nigrelli (1945) from *Rana clamitans* collected in New York, Connecticut, New Jersey and Pennsylvania. The morphology of *Trypanosoma rotatorium* is shown in Figure 1. *Rana clamitans* was also found to be infected with at least 5 other different and discrete morphological types of trypanosomes. The incidence and intensity of infection of these other types were low, and they will not be discussed in this report.

The parasitemia levels and incidence in frogs showing the presence of circulating blood *T. rotatorium* are indicated in Tables 1 and 2 and Figure 2. The difference in the parasitemia level of frogs captured in the late summer (17 August 1966) when compared to the parasitemia level of frogs captured in the winter were highly significant (Table 2). Table 3 shows that the sex of the frog does not influence the parasitemia

TABLE 1

Seasonal variation in the percentage of *R. clamitans* with *T. rotatorium* in the peripheral blood.

Date Frogs Collected	Number of Frogs Collected[1]	Percentage of Frogs with *T. rotatorium* in Peripheral Blood	Water Temperature °C
2 Aug. 65	3	100	ND[2]
27 Aug. 65	10	100	26.5
30 Sept. 65	15	93	ND
27 Oct. 65	23	78	15.0
3 Nov. 65	29	76	ND
17 Nov. 65	17	59	15.0
1 Dec. 65	6	66	10.0
6 Jan. 66	23	13	15.0
24 Feb. 66	7	0	9.5
10 Mar. 66	71	66	14.0
17 Mar. 66	28	93	19.0
24 Mar. 66	16	94	15.0
1 April 66	20	100	ND
14 April 66	14	93	20.0
12 May 66	21	90	23.5
9 June 66	2	100	ND
8 July 66	17	100	28.0
1 Aug. 66	13	100	28.0
10 Aug. 66	29	97	26.5
17 Aug. 66	30	100	27.0
27 Sept. 66	36	100	24.0
7 Nov. 66	16	100	24.0
30 Jan. 67	10	0	ND
Totals	456	—	—
Average	—	81	20.0

[1] All frogs greater than 5.0 cm in length.
[2] not determined

levels. Frogs of both sexes showed approximately equal parasitemia levels throughout the year (probability $< 10\%$). Preliminary data in Table 4 suggest that the age (size) of the frog does influence the parasitemia level. Frogs 4.0 cm or less in length were generally negative for trypanosomes, whereas a higher percentage of frogs above 4.1 cm captured at the same period showed detectable circulating trypanosomes. In frogs above 5.1 cm, there was no significant increase in parasitemia level or percentage of infected frogs with increase in age (size).

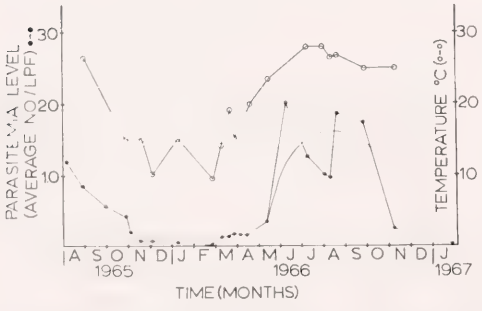

Figure 2. Seasonal variation in the blood parasitemia level of *Trypanosoma rotatorium* from *Rana clamitans*.

DISCUSSION

It appears that the parasitemia level of *Trypanosoma rotatorium* in *Rana clamitans* varies seasonally. The parasitemia level or seasonal cycle is not influenced by the sex of the frog. Additionally in frogs over 5.1 cm long the age (size) of the frog had little apparent relation to the parasitemia level or seasonal cycle. The seasonal cycle may be correlated with the water temperature from which the frogs were captured (Figure 2). Frogs captured in the field during the end of January and February were consistently negative for circulating blood trypanosomes. These same frogs when brought into the laboratory became positive at approximately the same time that the incidence and intensity of infection increased in other frogs obtained from the field. These

TABLE 2
Seasonal variation in the blood parasitemia level of *T. rotatorium* from *R. clamitans*.

Date Frogs Collected	Number of Frogs Collected[1]	Average Parasitemia Level (No./LPF)	Range	Probability (%)[2]
2 Aug. 65	3	1.20 ± 0.90	0.2–2.0	NS[3]
27 Aug. 65	10	0.81 ± 0.53	0.3–2.0	> 0.1
30 Sept. 65	15	0.59 ± 0.56	0–2.0	> 0.1
27 Oct. 65	23	0.43 ± 0.58	0–2.0	> 0.1
3 Nov. 65	29	0.20 ± 0.31	0–1.5	> 0.1
17 Nov. 65	17	0.06 ± 0.05	0–0.1	> 0.1
1 Dec. 65	6	0.07 ± 0.05	0–0.1	> 0.1
6 Jan. 66	23	0.01 ± 0.04	0–0.1	> 0.1
24 Feb. 66	7	0	0	> 0.1
10 Mar. 66	71	0.13 ± 0.28	0–2.0	> 0.1
17 Mar. 66	28	0.12 ± 0.08	0–0.5	> 0.1
24 Mar. 66	16	0.21 ± 0.18	0–0.6	> 0.1
1 April 66	20	0.19 ± 0.12	0.1–0.5	> 0.1
14 April 66	14	0.16 ± 0.16	0–0.6	> 0.1
12 May 66	21	0.33 ± 0.48	0–2.0	> 0.1
9 June 66	2	2.00 ± 2.70	0.1–3.9	NS
8 July 66	17	1.24 ± 1.94	0.1–7.8	NS
1 Aug. 66	13	1.10 ± 0.62	0.1–2.1	> 5
10 Aug. 66	29	0.96 ± 0.71	0–2.3	> 2
17 Aug. 66	30	1.75 ± 1.00	0.1–3.7	—
27 Sept. 66	36	1.77 ± 1.21	0.2–4.5	NS
7 Nov. 66	16	0.24 ± 0.16	0.1–0.6	> 0.1
30 Jan. 67	10	0	0	> 0.1

[1] All frogs greater than 5.0 cm in length.
[2] Based on student's "T" test. The parasitemia levels of frogs captured on 17 August 1966 were compared to the parasitemia levels of frogs captured at other times of the year.
[3] Not statistically significant ($P = < 10\%$)

results are preliminary and require further verification, however, they do indicate (1) that negative frogs probably are not truly negative but harbor low undetectable numbers of trypanosomes; and (2) that temperature is not solely responsible for the variable parasitemia levels, since the laboratory temperature was relatively constant and warmer than field conditions. The frog seasonal mating cycle and/or seasonal changes in photoperiod may play a role in controlling intensities of infection. Both of these possibilities are currently under investigation.

There have been few epidemiological studies on trypanosome infections of frogs. Diamond (1965) reported that 27% of *Rana pipiens* collected from Anoka and Ramsey County, Minnesota were infected with *Trypanosome pipientis*. Diamond also reported a 38.5% infection of *R. pipiens* from Anoka County, Minnesota collected on the 22 July 1949. These percentages are considerably lower than those reported here, and suggest that the incidence of infection and intensities of infection by frog trypanosomes may vary according to the geographical area and time of the year in which the frogs are examined. The presence of a 24-hour cycle influencing varied parasitemia levels of *T. rotatorium* in *R. clamitans* has been described (Southworth, *et al.*, 1968), and suggests that the time of day must also be considered in any analysis. In other words, to compare any two host populations of one species in similar climatic areas for the incidence and intensity of infection it would be necessary to examine all hosts at comparable daily and seasonal times.

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TABLE 3

Sex and seasonal variation in blood parasitemia level of *T. rotatorium*.

Date Frogs Collected	Male Frogs			Female Frogs		
	Number of Frogs Collected[1]	Percentage of Frogs with *T. rotatorium* in Peripheral blood	Average Parasitemia Level (No./LPF)	Number of Frogs Collected	Percentage of Frogs with *T. rotatorium* in Peripheral blood	Average Parasitemia Level (No./LPF)
6 Jan. 66	6	0	0	17	18	0.02 ± 0.04
10 Mar. 66	20	70	0.17 ± 0.43	51	65	0.12 ± 0.15
17 Mar. 66	11	91	0.15 ± 0.13	17	94	0.11 ± 0.05
24 Mar. 66	4	75	0.13 ± 0.13	12	100	0.23 ± 0.19
1 April 66	3	100	0.17 ± 0.12	17	100	0.19 ± 0.12
14 April 66	5	100	0.16 ± 0.14	9	89	0.17 ± 0.17
12 May 66	4	75	0.13 ± 0.13	17	94	0.38 ± 0.49
8 July 66	8	100	1.83 ± 2.71	9	100	0.76 ± 0.66
1 Aug. 66	6	100	0.90 ± 0.40	7	100	1.28 ± 0.75
10 Aug. 66	11	100	0.92 ± 0.62	18	94	0.98 ± 0.77
17 Aug. 66	17	100	1.62 ± 1.04	13	100	1.95 ± 0.96
27 Sept. 66	15	100	1.65 ± 1.15	21	100	1.83 ± 1.25
7 Nov. 66	2	100	0.20 ± 0.14	14	100	0.24 ± 0.18
Totals	112	—	—	222	—	—
Average	—	86	—	—	84	—

[1] All frogs greater than 5.0 cm in length.

TABLE 4

Body length and blood parasitemia level of *T. rotatorium* and *R. clamitans*.

Date Frogs Collected	Body Length[1]											
	4.0 cm or less			4.1–5.0 cm			5.1–6.0 cm			6.1–7.0 cm			7.1–8.7 cm		
	No[2]	%[3]	$\bar{x}$[4]	No	%	$\bar{x}$	No	%	$\bar{x}$	No	%	$\bar{x}$	No	%	$\bar{x}$
10 Mar. 66	9	11	0.01 ± 0.04	13	31	0.04 ± 0.07	47	62	0.11 ± 0.15	22	77	0.20 ± 0.20	2	50	0.13 ± 0.17
24 Mar. 66	2	0	0	1	0	0	10	90	0.13 ± 0.09	5	100	0.30 ± 0.24	1	100	0.5
1 April 66	1	0	0	1	100	0.1	8	100	0.18 ± 0.14	12	100	0.09 ± 0.12	0	0	0
12 May 66	1	0	0	9	78	0.46 ± 0.6	7	86	0.17 ± 0.11	11	91	0.27 ± 0.33	3	100	0.82 ± 1.02
17 Aug. 66	0	0	0	2	100	2.5 ± 0.86	18	100	1.63 ± 1.02	12	100	1.98 ± 1.03	0	0	0
27 Sept. 66	0	0	0	11	100	1.45 ± 1.02	28	100	1.82 ± 1.17	7	100	1.86 ± 1.45	1	100	1.2
Totals	13	—	–	37	—	—	118	—	—	72	—	—	7	—	—
Average	—	7.8	–	—	68	—	—	83	—	—	92	—	–	86	—

[1] Body length measured from tip of snout to vent.
[2] Number of frogs collected.
[3] Percentage of frogs with *T. rotatorium* in Peripheral Blood.
[4] Average parasitemia level (No./LPF).

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