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PRELIMINARY OBSERVATIONS OF FROG FILARIASIS [1] IN NEW JERSEY [2]

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Abstract

New Jersey bullfrogs, *Rana catesbeiana,* were found to be heavily infested with filarial worms of the genus *Foleyella* Seurat 1917. A technique was devised to rapidly screen amphibians for the infection. The results of a state-wide survey are presented and a possible life cycle is suggested. Preliminary transmission studies indicated the mosquito, *Culex territans,* to be a natural vector.

Introduction

Blood samples collected for serological studies in 1966 revealed that bullfrogs, *Rana catesbeiana,* in certain areas of New Jersey were circulating high levels of microfilariae. Dissections performed on infected frogs revealed a filarial worm of the genus *Foleyella* Seurat 1917 residing in the body cavity of the host. Both male and female worms were found encsyted in intestinal mesentery, encysted in mesentery about the kidneys, or moving freely upon the liver and spleen of the host.

Members of the genus *Foleyella* characteristically reside in the abdominal cavity of a herptilian host and produce sheathed microfilariae which circulate in peripheral blood. Four species in the genus have been reported from North American frogs. The discovery of *Foleyella ranae* encysted in the mesenteries of a Louisiana bullfrog, *Rana catesbeiana,* and detection of *Foleyella americana* in leopard frogs, *Rana pipiens,* from Illinois, afforded the first North American reports of species in this genus (Walton, 1929). In 1939, Wehr and Causey described two new members of the genus *Foleyella brachyoptera* and *Foleyella dolichoptera,* from southern leopard frogs, *Rana sphenocephala,* collected in Florida. *Foleyella dolichoptera* appears to be a synonym for *Foleyella leiperi* (Raillet, 1916) also collected in Florida and redescribed by Cowper (1946). *Foleyella ranae, F. brachyoptera* and *F. dolichoptera* syn. *leiperi* have been shown to develop to the infective stage in mosquitoes (Causey, 1939; Kotcher, 1941).

In 1968, research was initiated to investigate the identification, distribution and life cycle of *Foleyella sp.* in New Jersey. Studies on mosquito feeding habits (Crans, 1964) had revealed that one particular species, *Culex territans,* fed essentially on frogs and was known to be common in areas where the infection occurred. Investigating the possible involvement of this mosquito as a natural vector of the parasite was a major objective of this research.

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[1] *Foleyella sp.* (Nematoda: Filaroidea).

[2] Paper of the Journal Series, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey 08903.

Materials and Methods

Filarial Identification

Infected frogs were pithed and dissected so that adult filarial worms could be obtained for identification. Adult worms were carefully teased from the mesenteries and placed alive in normal saline. Specimens were then heat relaxed and placed in 70% ethyl alcohol with 10% glycerin added to maintain relaxation. Females were dissected under stereoscopic magnification so that measurements of internal structures could be obtained. Males were preserved in Turtox CMC mounting media to make anal papillae more evident and to make spicule measurements possible.

Survey

Frogs were captured throughout the state and brought to the laboratory for observation. Each frog was arbitrarily assigned a year class on the basis of size at the time of capture according to growth rates posed by Raney and Ingram (1941) and Ryan (1953). After each specimen was identified, sexed and measured, a blood-smear was taken for permanent record (Fig. 1). A rapid screening technique for microfilarial activity was devised so that large numbers of frogs could be processed.

The method employed the Unopette[3], normally used for erythrocyte counts but modified somewhat for this study. Basic components of the Unopette included a plastic reservoir containing a pre-measured volume of diluent and an attachable capillary assembly (Fig. 2). The reservoirs in this study contained 0.5 ml physiological saline with no preservative added. The preservative added to commercial preparations was found to kill the microfilariae. Capillary assemblies consisted of uniform-bore glass tubes available in volume from 1 to 50 μl. Each unit was equipped with an overflow chamber, handle and shield. A 5 μl capillary was selected and a uniform blood dilution of 1:100 was thereby secured from each frog tested.

Frogs were lightly toe-clipped to initiate blood-flow and 5 μl of peripheral blood drawn into the assembly by capillary attraction. The sample was then diluted in the plastic reservoir and transferred to a Scott hookworm counting slide for examination under stereoscopic magnification. The frog erythrocytes were quite heavy and quickly settled to the bottom. Living microfilariae settled more slowly and were evident in the sample when viewed at 80X with light reflected from beneath. The active movements of microfilariae made even a single specimen instantly noticeable in this sample. Careful manipulation of the light source revealed numerous trypanosomes in most samples.

Microfilariae could be further studied if acetic acid was substituted for saline in the Unopette reservoir. Acetic acid not only destroyed the erythrocytes but killed and straightened microfilariae for counting or comparative measurements.

Developmental Stages in Mosquito Hosts

Culex territans were collected as larvae in the field and allowed to pupate and emerge in small cages. The specimens readily fed on infected frogs in the laboratory and were thereafter maintained on sugar water until dissections could be performed. Infected mosquitoes were dissected in saline and examined for developing stages of the parasite.

Results and Discussion

Filarial Identification

Twenty-five bullfrogs circulating microfilariae were sacrificed and examined for nematodes during the summer of 1968. A mean of twelve worms was found in the twenty-five frogs dissected; male filariae outnumbered females by a ratio of 7:5. A maximum of twenty-

[3] Disposable Blood Diluting Pipette — Becton, Dickinson and Company, Rutherford, New Jersey.

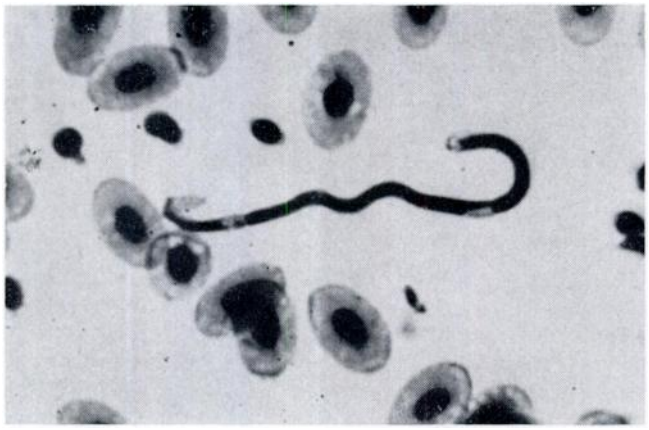

FIGURE 1. *Giemsa stained microfilaria from peripheral blood of bullfrog.*

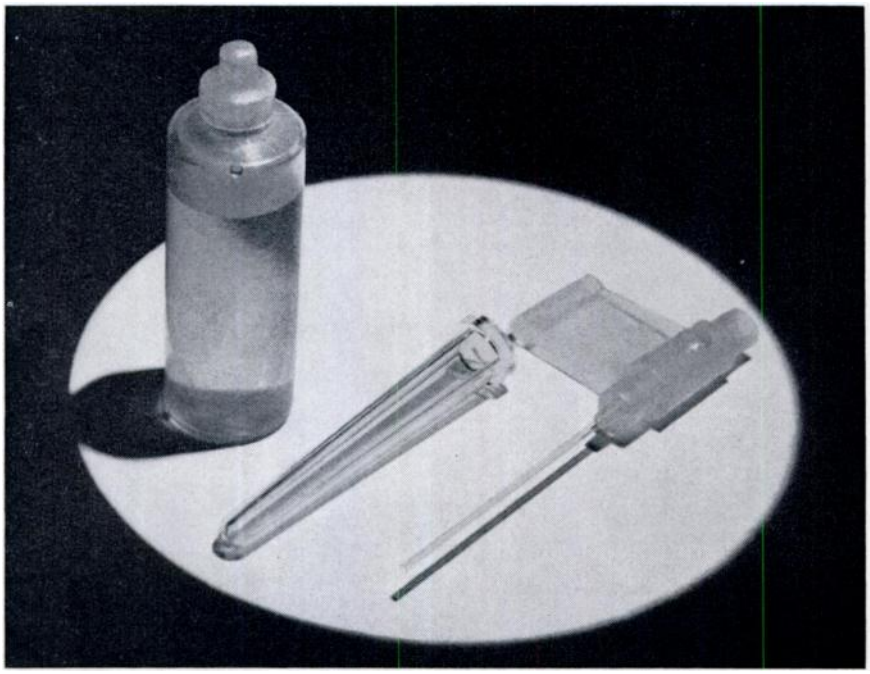

FIGURE 2. *The Unopette, showing sealed reservoir containing premeasured diluent and capillary assembly removed from shield.*

nine adult worms was found in a single frog, seventeen of which were males and twelve females.

Adult female *Foleyella sp.* measured 30-40 mm. in length and 700-800 μ in width. All specimens possessed a pre-esophageal vulva. Adult male *Foleyella sp.* measured 16-20 mm. in length and 400-475 μ in width. Five pair of fleshy post-anal papillae were evident. Microfilariae measured 90-120 μ in length and 3.0-4.0 μ in width. All specimens examined appeared to belong to a single species.

New Jersey *Foleyella* did not accurately fit available descriptions for any reported North American species in the genus. Specimens most closely resembled *Foleyella brachyoptera* Wehr and Causey but until type specimens can be obtained for comparison, conclusive identification will not be proposed.

Survey

A total of 232 bullfrogs were examined for microfilariae from fifteen different New Jersey ponds during 1968 (Table 1). Although other frog species were also examined, only bullfrogs showed circulating microfilariae. The infection appeared to be statewide, but bullfrogs captured north of the coastal plain were more frequently found harboring the parasite. As high as 70% of frogs in certain ponds were circulating microfilariae. By contrast, only two specimens captured in the acid waters of Southern New Jersey were found infected. No explanation for this apparent geographic distribution can be offered at this time.

Microfilarial activity in the blood was directly correlated with age of the frog in the northern portion of the state. Only a single frog in its first full year of growth was found circulating the parasite. This specimen was captured from an overcrowded pond and could have been a stunted individual already in its second full season. Twenty-one percent of those frogs tested in the second year class showed evidence of the infection and more than 50% of frogs three years old and older circulated microfilariae. Upon emergence from hibernation in the spring only frogs in the third year class showed evidence of the parasite. It was not until May that infected individuals in their second year of growth were found.

It would appear from these data that the life cycle of the parasite spans several years and that repeated exposure merely enhances the chance of contracting the infection, however, information gained from early and late season dissections largely discredited this theory. Bullfrogs collected in late August and September frequently demonstrated circulating microfilariae but dissections revealed that mature filariae were either absent or in a state of decay. Only immature forms were found encysted in mesentery and minute immature specimens were found embedded in the liver, spleen and kidneys. These findings sug-

TABLE 1. *Microfilarial Incidence in 232 Bullfrogs,* Rana catesbeiana, *sampled from fifteen New Jersey ponds during 1968.*

	1st Year Class[1]			2nd Year Class[2]			3rd Year Class[3]		
AREA	No. Examined	Positive No.	%	No. Examined	Positive No.	%	No. Examined	Positive No.	%
Northern New Jersey	18	1	5.5	76	16	21.0	89	46	51.6
Southern New Jersey	9	0	0	19	0	0	21	2	9.5

[1] Bullfrogs in first full year of growth.
[2] Bullfrogs in second full year of growth.
[3] Bullfrogs in third full year of growth and older.

gested that sexually mature filariae died before the frog entered hibernation and only immature forms and microfilariae overwintered in the host. Immature forms encysted in mesentery probably represented infections sustained early that same year. Minute forms found in organs resembled infective stage larvae and were more likely only recently introduced into the host.

Several frogs dissected soon after emergence from hibernation in the spring of 1968 contained no adult worms even though microfilariae were evident in the blood. It can be assumed that these frogs harbored mature adults during the previous summer. These died in the fall but microfilarial activity was maintained through the winter. These few frogs had apparently not been re-infected in 1967 and, therefore, harbored no adults when captured. The actual infective bite in these cases would have been sustained two years prior in 1966.

In view of the fact that only frogs in their third year of adult life showed microfilarial activity early in the season, all parasites circulating at that time were most likely the progeny of adults injected as infective larvae two years prior. Frogs in their second year of growth examined early in the season may well have contained maturing worms received the previous summer but were not yet circulating microfilariae. Two years prior these small frogs would have spent the majority of the season in the tadpole stage and infection at this time would have been unlikely.

These findings suggest that sexual maturity of the parasite apparently takes nearly a full year in the frog. Since bullfrogs enter hibernation in November and do not emerge until late March in New Jersey, five months of the parasite maturation period is spent in hibernation. These adult worms produce microfilariae until late summer thus the entire life span from infective larva to senility appears to approach fifteen months in New Jersey.

Considerable speculation has been posed regarding the activities of filarial parasites immediately after introduction to the host. On three occasions infective stage larvae were detected in the peripheral circulation of frogs. These were first thought to be representatives of a second, very large species of microfilaria but mounted specimens revealed a developed alimentary tract as well as other structures common with those of forms taken from the organs of frogs about to enter hibernation. This would indicate that infective larvae may enter the circulatory system soon after gaining entrance into the host and circulate in the blood until filtered out by the liver, spleen and kidneys. These specimens may later gain entrance to the abdominal cavity through these organs.

Developmental Stages in Mosquito Hosts

The microfilariae of *Foleyella sp.* readily passed through three developmental stages in a native strain of the amphibian feeding mosquito, *Culex territans.* Previous investigations regarding developmental studies in Culicidae utilized laboratory strains of *Culex pipiens* and *Aedes aegypti.* These mosquito species do not normally accept amphibian hosts in nature and considerable difficulty was experienced by investigators inducing the mosquitoes to accept a blood-meal from a frog. In addition, considerable mosquito mortality was reported during the 18-20 day interval between ingestion of microfilariae and appearance of infective stage larvae.

In preliminary developmental studies with *Culex territans,* mortality was negligible and infective stage larvae were detected within ten days. Developmental stages appeared to occur throughout the hemocele of the mosquito with a concentration of specimens in abdominal fat body. In one extreme case, as many as one hundred infective larvae were dissected from a single mosquito nine days after an infective blood-meal. Many of these were already located in the proboscis and head capsule; others were freely moving in and out of the coxal cavities of the legs.

Summary and Conclusions

An as yet unidentified filarial worm in the genus *Foleyella* is a common parasite of New Jersey bullfrogs. A single species is thought to be widespread in the state. A tentative life cycle of the parasite may be postulated on the basis of observations made during 1968.

The adult worm resides in the body cavity of the frog encysted in intestinal mesentery and mesenteries about the kidneys. Adult worms produce sheathed microfilariae which circulate in peripheral blood. Microfilariae are capable of completing development to the infective stage in the amphibian feeding mosquito, *Culex territans*. Infective larvae are thought to be transmitted to frogs in nature by the bite of this mosquito. Infective larvae may directly enter the circulatory system of the host and circulate until filtered out by the liver, spleen and kidneys. Immature forms and microfilariae overwinter in the amphibian host; sexually mature adult filariae apparently die before the frog enters hibernation. Frogs emerge in the spring circulating microfilariae from a prior year's infection. It is not known how long microfilariae circulate in the absence of mature adult worms. Adult worms reach sexual maturity in the frog during the spring and produce microfilariae until senility is reached late in the summer. The entire life span from infective larva to senility appears to approach 15 months in New Jersey.

It would appear that the potential of frog filariasis should be further investigated. This readily available filarial parasite coupled with an easily reared intermediate vector shows great promise as a model to study the mechanism of filarial transmission in the laboratory.

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