STAINING TECHNIQUES TO FACILITATE COUNTS OF ABOMASAL PARASITES

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Abstract: The Abomasal Parasite Count (APC) provides an effective yet time-consuming technique for assessing parasite burdens in southeast white-tailed deer *(Odocoileus virginianus)* herds. Staining techniques utilizing Rose Bengal to facilitate parasite identification are described. Total water hardness of 74 mg/l or greater is essential to the proper application of these staining techniques. A concentration of 1% Rose Bengal stain yields a considerable savings of laboratory time. Expense of the technique is minimal at a cost of 4 cents per sample. Guidelines for the most effective use of this staining method are provided.

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An ability to provide qualifying values derived from scientifically accepted techniques has aided many states in establishing more contemporary wildlife management programs. The era of intuitive assessment and subjective wildlife management has passed, due to a better educated and more demanding public. The APC, developed by the Southeastern Cooperative Wildlife Disease Study (Eve and Kellogg 1977), is a technique which provides the southeastern United States a better tool for evaluating and managing white-tailed deer populations.

The APC provides insight into the critical relationship of a deer herd to the present carrying capacity (Eve and Kellogg 1977) as well as indicators of overall physical condition (Osborne et al. 1979). The Florida Game and Fresh Water Fish Commission utilizes APC's on a statewide basis, and in the 1980 - 81 hunting season, over 400 abomasa were collected for analysis. Manpower expenditures exceeded 1000 man-hours to prepare, count and summarize the data collected for dissemination and use by field personnel. The purpose of this paper is to describe a staining technique for APC's which appears to increase accuracy of the count while significantly reducing manpower expenditures in the laboratory. We wish to thank Mr. Grady Lea of the Regional Medical Laboratories, Pensacola, Florida, for his assistance in testing various stains and in water chemical analysis. Appreciation is also extended to the many individuals in the Division of Wildlife, Florida Game and Fresh Water Fish Commission for their contributions in developing this technique.

METHODS

Materials used to collect, preserve, and analyze abomasal parasites have been described by Eve and Kellogg (1977). Abomasal samples were obtained from hunter-harvested deer collected throughout Florida during the 1980 - 81 season. Carbol Fuchsin and Rose Bengal stains, considered rapid types (staining within 24

hours), were used in this study. All other stains available were considered longer acting stains and were not utilized. Analysis of water available at the 5 sites where samples were examined in this study was provided by the Florida Department of Environmental Regulation. Additional materials used to accelerate or enhance the staining process were distilled water, phenol, calcium chloride, and liquid chlorine bleach.

Stains and additives were incorporated into individual samples at 1 of 2 points in the APC procedure. The 1st method involved the addition of 3 ml of commercial stain into the 3.8 liter (gallon jug) container. The stained solution was allowed to stand for 24 hours or more and the entire sample was then strained through a No. 100 sieve and washed. Fifty-milliliter aliquots were then taken from the stained and washed sample and counted according to standard APC procedure.

The 2nd method involved straining and washing the unstained abomasal sample. The sample was then placed into a graduated Erlenmeyer flask and filled to 1000 ml with fresh water. A 50-ml aliquot was taken from this sample according to APC procedure. The 50-ml sub-sample was placed in a small container and 1 ml of stain was added; an additional 50 ml of fresh water was added to the container via washing the aliquot device to ensure all parasites were included into the sample. The container was allowed to stand 24 hours or more before analysis.

RESULTS AND DISCUSSION

Between February and May 1981, 300 abomasal samples were stained using Rose Bengal and Carbol Fuchsin. Rose Bengal stain proved to be the far superior stain both in speed and color. Mason and Yevich (1967) indicated similar results with benthic samples claiming, "Rose Bengal stain was rapid and provided a vivid red color for identification purposes." Helminths stained with Carbol Fuchsin took from 30 to 36 hours or longer to stain, and the color was not as bright, regardless of increasing amounts of Carbol Fuchsin stain added to the sample.

Water chemistry (Table 1) appeared to be the most significant factor involving uptake of the stain into the parasite. Phenol and distilled water did not influence the staining process. Liquid chlorine bleach, however, disrupted the staining process by bleaching available stain within the parasite and water. The addition of calcium chloride promoted staining, apparently due to the increase of total water hardness.

A complex of chemical properties of the water appears to affect the staining process. Total hardness promotes staining as hardness increases (Table 1). In contrast high chloride values and lower total hardness values will neutralize the staining process. Best results were obtained at locations with total hardness values >74 mg/l and chloride values <2.7 mg/l (Table 1). Total hardness appeared to have an overriding effect on chloride where extremely high hardness values negated the effect of higher chloride values (Table 1, column 3).

Samples prepared by the 2nd method, utilizing Rose Bengal stain, provided better economy to the APC technique. The authors feel a greater accuracy due to better visibility is obtained, especially in identifying the genus *Trichostrongylus*, when utilizing this stain technique; however, this claim cannot be substantiated. Samples can be counted in 15 to 30 min utilizing this method, representing a

Table 1. Chemical analysis (mg/l) and pH of tap water available at 5 Florida locations utilized for staining experiments on abomasal parasites.

Chemical Character	Niceville[a]	DeFuniak Springs[a]	Ocala[a]	Blackwater Fish Hatchery	Panama City
Calcium	24.0	20.0	7.0	6.2	27.5
Magnesium	14.0	5.9	11.0	3.3	17.0
Potassium	3.2	0.6	0.9	4.7	1.9
Bicarbonate	164.0	81.0	146.0	164.0	124.0
Sulfate	7.8	5.9	90.0	6.0	19.0
Chloride	2.8	2.7	16.0	5.5	81.0
Total hardness	120.0	74.0	220.0	41.0	162.0
Sodium	8.3	2.1	10.0	20.1	28.0
pH	7.7	7.9	9.4	7.2	7.8

[a] Locations where acceptable stain was achieved (without additives) within 24-hour period.

minimum time savings of 50%. Cost per sample using Rose Bengal approaches $0.04 (Method 2) and $0.12 (Method 1).

The use of Rose Bengal can be most effective if the following Method 2 guidelines are followed: concentration of stain should equal or exceed 1% of the sample to be counted; total water hardness should equal or exceed 74 mg/l (hardness may be increased by the addition of calcium chloride); use a microscope with a bright light source directed from below the petri dish; and dilute the sample sufficiently to avoid parasites being concealed in other matter.

LITERATURE CITED

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