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WHIRLING DISEASE *Myxosoma cerebralis* SPORE CONCENTRATION USING THE CONTINUOUS PLANKTON CENTRIFUGE

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Abstract: A method for concentrating and detecting the spores of the cause of whirling disease (*Myxosoma cerebralis*) of salmonids is described. The method involves homogenization of head skeletons, screening out tissue shreds, and concentrating with a continuous plankton centrifuge.

INTRODUCTION

The detection of whirling disease spores in carrier fish has always presented the biologist with diagnostic problems.[2] Since new disease regulations may require the diagnosis of whirling disease in large lots of trout,[3] practical methods for concentrating spores should be available to the fishery pathobiologist.

At the Benner Spring Fish Research Station a procedure using the continuous plankton centrifuge[1] (Figure 1) has been developed to concentrate whirling disease spores from fish in research experiments and production lots. This method has been used for 4 years and has given satisfactory results in the quantitative studies carried out in our whirling disease research projects. Rainbow trout, for example, which do not exhibit signs of the disease, have been shown by this method to harbor relatively large numbers of spores.

FIGURE 1. The plankton centrifuge removes the excess water from the filtrate through the centrifugal force created by the revolving drum. This excess water is carried away through a rubber tube situated below the drum. Most solid particles are deposited on the wall of the revolving drum in the first centrifuge run.

MATERIALS AND METHODS

The materials needed are: Waring blender, blender bottle (175 ml or larger), membrane filter apparatus, wire

[1] Plankton centrifuge manufactured by G. M. Mfg. and Instrument Corporation, P.O. Box 326, Nanuet, New York 10954.

[2] For more critical work one may screen the homogenate through graded mesh-size screens, e.g., 520 - 260 - 120 nm mesh.

mesh prefilter screen—mesh size 725 mm,[2] continuous plankton centrifuge, separatory funnel and ring stand, 27 ml specimen bottle, hemocytometer.

The fish are decapitated near the base of the skull. Larger fish are best handled by heating the heads at 45 C for 2-3 min and then removing as much soft tissue as possible. Large pieces may be split or cut into smaller pieces.

STEPS

1. Take five to ten heads of 8-15 cm fish or one to five heads from larger fish and blend for 5 min in 175-200 ml of water. If spores are intended for infection experiments, chlorinated water should be avoided.
2. Remove the blended material and vacuum filter the entire volume through a membrane bacteriological filter using a wire mesh prefilter. The filter may clog and should be cleaned with water. The rinse water becomes part of the filtrate to be saved. The procedure removes fish scales and other coarse material which is discarded.
3. Place the entire amount of filtrate in a separatory funnel discharging into the plankton centrifuge. Set a low flow from the separatory funnel into the centrifuge and run centrifuge at high speed.
4. Centrifuge until all water is removed. The residue adhering to the walls of the revolving drum will contain spores and some fish tissue. Scrape residue from the walls of the plankton centrifuge revolving drum with a rubber policeman. Be sure to get all material.
5. Place the residue and suspended material from the drum into the 27 ml bottle and fill to the top with distilled water. Place the cap on the bottle and shake until the material is uniformly suspended (2 min).
6. Place a small amount of suspension under a coverslip of the hemocytometer if a spore count is desired.[6] Count the spores under four 1 mm^2 squares. We use four chambers, counting a total of 16 squares.

   Formula:

   $$\frac{\text{number of spores}}{1\text{ ml}} = \frac{\text{total number of spores counted} \times 10^4}{\text{number of 1 mm}^2\text{ areas counted}}$$

   27 ml (Suspension Volume) x number of spores in 1 ml = total number of spores.
7. Equipment should be scrubbed thoroughly, soaked in half-strength household bleach (NaOCl) for 10 min and rinsed thoroughly after each use.

PLANKTON CENTRIFUGE EFFECTIVENESS

Using this technique we have recovered 3.4 x 10^4 to 9 x 10^6 from our pooled samples during the past 4 years. G. L. Hoffman recovered 4.2 x 10^5 to 4.3 x 10^6 spores per fish from heavily infected fish using this technique.[3] The late W. Tidd also obtained good results with the plankton centrifuge.

To confirm the effectiveness of the plankton centrifuge in spore concentrating, a test was devised to determine the relative loss of spores in the water which is removed from the centrifuge. Five heavily infected rainbow trout heads were run through the spore concentration procedure which consisted of three trial runs, reusing the same suspension.

[3] Hoffman, G. L. 1974. Personal communication. Eastern Fish Disease Laboratory, Kearneysville, West Virginia 25430.

Spores from the first run were concentrated and the number per ml estimated. The water from the first run was collected and recentrifuged, and the number of spores per ml of suspension was estimated for the second time. The water from the second run was collected, recentrifuged, and again the spores per ml of suspension was estimated. By this time the spore count was very low and further concentration was believed unnecessary.

The estimated spore number from each run was added and total spore count obtained (Table 1).

To determine the absolute loss of spores after the first centrifuge run, a seed population of 10,250,000 spores was added at step three of the procedure. An estimated recovery of 8,370,000 spores was achieved, which indicates an 83% efficiency of recovery.

TABLE 1. Spores recovered from the same suspension in three runs.

	Estimated Spores Per ml	Percentage of Total Spores Recovered
Run #1	458,750	96.2
Run #2	17,500	3.7
Run #3	630	0.1
TOTAL	476,880	

DISCUSSION

Because the spores are trapped in skeletal structures and granulomas they are difficult to detect in carrier fish. Recent studies on spore concentration methods include blending, filter paper filtration and centrifugation,[6,8] and fish digestion.[1,4,5,7] The plankton centrifuge concentrates whirling disease spores effectively for diagnostic purposes. It has the advantage of speed and simplicity and the final suspension is fairly debris-free. Relatively few spores are lost after the first centrifuge run, thus a reasonable quantitative estimate can be made and the disease level of the population determined.

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