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A NEW PRACTICAL PROCEDURE FOR THE DETECTION OF *SPHAEROSPORA* (MYXOSPOREA) BLOOD STAGES

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Sphaerospora renicola, a parasite of the common carp, has three distinct developmental stages (Csaba et al., 1984). The first stage is found in blood plasma (Csaba, 1976). The second stage is the presporogonic form found in the swimmbladder and the third stage is the spore stage developing in the renal tubules. The blood stages are observed either in fresh drops of blood on the basis of the parasites' characteristic dancing motion, or in Giemsa stained smears. Grupcheva et al. (1985) and Odening et al. (1989) used the latter approach for surveying the seasonal incidence of *Sphaerospora* blood stages. However, the sensitivity of this technique is questionable and it is probable that low-level infections may be overlooked.

For more reliable diagnosis of low-level infections of the parasites in small-volume samples, we have applied an enrichment method which is described in this paper.

Material and methods:

Parasite survival in blood samples from common carp, *Cyprinus carpio* L. was studied at room temperature and at 0°C. For this purpose, motile stages in the blood were counted immediately before sampling and after incubation under anaerobic conditions.

A total of 85 carp fry, weighing 7 to 23 g were used for the test. The carp fry were obtained from ponds in which *Sphaerospora* infection had occured earlier.

From the caudal vein of each fish a maximum of 0.1 ml blood was taken by a tuberculin syringe containing 4-5 IU heparin. Parasites were counted in fresh preparations and Giemsa stained smears of blood, and enriched suspensions (see below). Thirty visual fields per preparation were examined at a magnification of x 400.

Parasite enrichment from the blood samples was done using microhematocrit tubes or agglutination tubes. A heparinized hematocrit tube was filled with blood to 40%, then, by interposing a small bubble, the tube was filled with Ficoll-Paque solution (Pharmacia Fine Chemicals, Uppsala, Sweden). Subsequently the tube was stoppered from the side of the Ficoll-Paque solution and centrifuged in a hematocrit centrifuge. Centrifugation was continued until the lymphocyte layer was sharply separated from the other blood elements at the density interface above the Ficoll-Paque solution. In our case, it took 30 seconds. Subsequently, the hematocrit tube was scratched and broken under the lymphocyte layer which was then examined microscopically.

In the method using agglutination tubes, 90 µl blood sample was layered onto 0.1 ml Ficoll-Paque solution (diameter: 5 mm) and centrifuged at 160 *g* for 10 minutes at 20°C. The cells situated at the density interface were collected, the suspension was made up with saline to three times the volume of the white cells pellet and centrifuged in a conical microtube at 200 *g* for 10 minutes at 20°C. The pellet was resuspended in 3 µl carp serum and checked for the presence of parasites.

The fish were killed after the blood sampling. The caudal sac of the swimbladder rich in capillaries and the choroid were placed under a coverslip and examined for the presence of protozoans showing their characteristic dancing motion in the capillaries.

Results

Incubation at room temperature for the two hours did not alter the number of motile parasites (Initial count: 4.75

per visual field; count after incubation: 4.80 per visual fields). At the same time, in the blood samples cooled to 0°C, no motile stages were demonstrable after 30 minutes.

After enrichment by the hematocrit tube method, the parasites were easier to recognize in fresh preparations then in stained smears. In the case of blood samples cooled to 0°C, the parasites were identifiable only in stained smears. Blood protozoans were demonstrated in 14 of 85 fish examined. In 4 of the 14 fish, the parasites were found in the blood before enrichment. In these samples the number of parasites increased 8-10 fold by the enrichment procedure. In the samples centrifuged in agglutination tubes, the parasites were enriched 12- to 30-fold.

Of the 14 fish found infected by the enrichment method, 12 specimens were shown to be infected with *Sphaerospora* blood stages by examining the swimbladder capillaries. However, in the choriod capillaries and the blood discharged from these capillaries, *Sphaerospora* blood stages were demonstrable in all 14 fish.

Discussion:

The results demonstrate that enrichment of heparinized blood samples by density centrifugation is suitable for detecting *Sphaerospora* blood stages that would remain unnotice by the rapid method used earlier. In microhematocrit tubes, the blood stages can be enriched -10 times their original counts. A more effecient enrichment shall be achieved if the blood volume is increased. The studies of Lom et al. (1985), Baska and Molnár (1988) and Hedrick et al. (1988) indicate that blood stages occur in all *Sphaerospora*-infected fishes. At a low-level of infection, these stages are difficult to find in blood smears. By the method described here, the chance of detection is greatly increased. Examination of the choriod of the eye also seems to be more succesful for identifying infected specimens than direct examination of the blood. However, the fish must be killed for this. A further draw-back of this method is that the anatomy of the eye varies between fish species and, consequently, the diagnostic suitability of the blood vessels of the eye may also be different. For the enrichment by centrifugation, blood samples necessary for testing can be taken several times without killing the fish and thus, the dynamics of *Sphaerospora* infection can be monitored. In our experience, live parasites are easier to find. We recommend sample processing without cooling on ice since long survival of the parasites at room temperature makes this possible. If the sample is cooled to 0 °C, detection of the parasites will be successful only in stained smears. The presented method is likely to be suitable for enrichment of the developmental stages of all *Sphaerospora*-type myxosporeans *(Leptotheca, Ceratomyxa)* the early developmental stages of which are as yet unknown.

Summary

During the ontogeny of *Sphaerospora* (Myxosporea) species, characteristic developmental stages appear in the blood of the infected fish. At a low intensity of infection, these blood stages are around the detection limit by direct examination of blood samples. The success rate of detection can be increased by examining capillaries of the swimbladder and the choriod. However, the best results were obtained by density centrifugation of heparinized blood on Ficoll-Paque solution. After centrifugation, the *Sphaerospora* blood stages were situated in the lymphocyte layer and their counts were 8 to 30 times higher than the pretreatment values.

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