Nonlethal clinical techniques used in the diagnosis of diseases of fish

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As an increasing number of people in the United States are keeping aquatic organisms, the inclusion of aquatic cases in routine veterinary practice has become more common. With a little basic knowledge and understanding of aquatic animals, practitioners can provide valuable diagnostic and therapeutic information to their clients with ailing pets. As with domestic animals, a complete and accurate history and thorough external examination are prerequisite to the selection of appropriate diagnostic techniques as well as the formulation of any management or therapeutic plan.

When advising a client on bringing a fish into the veterinary clinic, the practitioner should recommend that the fish be brought in one container and a sample of water in a separate container. The fish should be transported with a small amount of water in a sealed plastic bag or clear container and protected from temperature extremes. Because water conditions have a considerable effect on the health and well-being of aquatic animals, an in-house evaluation of water quality (eg, temperature, pH, ammonia, nitrites, nitrates, and salinity) is paramount to any clinical diagnostic evaluation. Thus, the separate water sample helps reduce any artifactual changes that might occur in the quality of the water sample caused by the presence of the fish during transit. This additional water may also be used to provide fresh water for the fish for its transport home with the client.

There are a number of nonlethal clinical techniques that can be used on a live fish that often yield valuable diagnostic information. Most of these techniques are inexpensive, easy to perform, and generally do not require any additional specialized equipment than would normally be on hand in a veterinary practice. These techniques include skin, fin, and gill biopsies; bacteriologic cultures of the gill or skin lesion; tissue and fluid aspiration; and radiography. Most of the biopsy and aspiration techniques can be performed on live fish without the use of anesthesia, although light sedation of the fish often simplifies the procedure, making the procedure more easily accomplished and less stressful on the fish. When handling fish, latex gloves rinsed of any powder should always be worn to decrease abrasive and drying trauma to the skin of the fish and decrease the potential transmission of zoonotic infections. Depending on the species of fish, the type of procedure, and the amount of time anesthesia is required, a variety of anesthetics can be administered by a number of routes, although the most common method for minor procedures is to directly immerse the fish in an aerated buffered anesthetic solution of tricine methanesulfonate (MS-222). Onset and duration of anesthesia in fish is dependent on water temperature as well as the species, size, fat content, and general health of the fish. As a general rule, sedation is reached within 3 to 5 minutes at 20 to 50 mg of MS-222/L of water, whereas surgical anesthesia is reached within 5 to 10 minutes at 50 to 100 mg/L of water. By continuously monitoring the fish for changes in respiratory rate, buoyancy, and behavior, the fish can be removed from the anesthetic solution when the desired depth of anesthesia is reached. A separate container of fresh aquarium water should always be available for immediate recovery of the fish when the procedure is finished or in situations of unintentional anesthetic overdose.

Procedures

Skin biopsy (or mucus smear)—The skin is the primary target organ for many of the infectious pathogens of fish, especially external parasites. Therefore, performing a skin biopsy is one of the most useful and common techniques for diagnosing ectoparasitic problems. A skin biopsy is performed by gently scraping, in a cranial-to-caudal direction, a small area or lesion on the surface of the fish with the edge of a microscope slide coverslip or other blunt instrument (Fig 1A). Care should be taken to use only a minimal amount of pressure to obtain this superficial scraping, because removal of scales or damaging deeper layers of the skin may result in secondary bacterial infections or osmoregulatory imbalance in the fish.

The mucus of the skin scraping should be immediately transferred to a drop of aquarium water (either fresh, brackish, or salt water depending on the species of fish, but not municipal water because it may contain substances that can rapidly kill many external organisms) on a glass microscope slide and a coverslip carefully applied. This wet mount should then be examined under the compound microscope for free-swimming, attached, or encysted protozoa; metazoan parasites; fungal hyphae; or bacteria.

Fin biopsy—A fin biopsy is performed by cutting a small piece of tissue from the peripheral edge of 1 of the fins or the tail (Fig 1B). This procedure is often less traumatic to the fish than a skin biopsy, because it generally results in a smaller wound. The fin specimen should be immediately transferred to a drop of aquarium water on a glass microscope slide, spread to its full extent, and a coverslip should be carefully applied. This wet mount should then be examined under the microscope for protozoan or metazoan parasites, fungal hyphae, or bacterial colonies.

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Sampling of gill or skin lesions for bacterial isolation—Samples for bacterial isolation and identification can be obtained from the external surface of the gills or skin (Fig 1C). Specimens can be inoculated on standard bacterial agars (tryptic soy agar, brain heart infusion agar, blood agar, salt agar, etc) for growth at room temperature (20°C), because most bacterial pathogens of fish have an optimal growth range between 18 and 25°C. However, identification of a definitive pathogen from the external surface of a fish may be unrewarding, because the skin and mucus harbor many secondary, opportunistic, and nonpathogenic organisms, which may make isolation of a specific bacterial pathogen difficult.

Gill biopsy—A gill biopsy is performed by inserting the tip of a fine pair of scissors into the branchial (gill) cavity behind the operculum (gill cover) and cutting off the distal ends of several of the primary gill lamellae (Fig 1D). Care should be taken not to cut the branchial or cartilaginous arch of the gills, and as only the tips of the primary lamellae are removed, only a minimal amount of bleeding should occur. The gill tissue should be immediately transferred to a drop of aquarium water on a glass microscope slide, the individual elements of the gill tissue separated by teasing the lamellae apart, a coverslip carefully placed over the tissue, and a small amount of aquarium water added to keep the tissue moist (Fig 2). This wet mount should then be examined under the microscope for external pathogens.

Fecal examination—Examination of fecal matter for internal parasites in fish is accomplished with the same techniques used in mammals. A fresh fecal sample may be collected with a pipette from either the bottom of the aquarium or hanging from the vent of the fish. If an appropriate sample cannot be acquired from the environment or if examination of a specific fish is desired, the application of gentle pressure on the sides of a netted fish often produces the desired sample. The fecal sample is then examined as a direct smear or processed by standard floatation or sedimentation techniques and evaluated for the presence of protozoa, parasite eggs, and larvae. Although it is generally impossible to identify specific parasites, this technique does provide useful information as to the types of parasites that may be present in the fish.

Blood sampling—Obtaining an appropriate blood sample nonlethally from small fish can be both difficult and traumatic and often results in severe hypovolemia. Larger fish may be bled with or without anesthesia directly from the heart or from the caudal vessels of the tail by use of a syringe and needle. Venipuncture of the caudal vessels of the tail can be accomplished via a...
ventral midline or lateral line approach. Fish blood coagulates rapidly; thus, the use of heparinized syringes and needles and avoidance of contamination by tissue fluid are necessary for obtaining a proper blood sample. Unfortunately, alterations of hematologic and serum biochemical values in most species of fish are difficult to interpret and are therefore of limited value. However, various blood protozoans can be identified using this technique.

**Tissue and fluid aspiration techniques**—Abdominal fluid or tissue aspirates can be obtained from fish by use of techniques similar to those used in mammals. General anesthesia greatly facilitates the acquisition of proper samples and greatly decreases the stress and potential for injury to the fish. To sample for possible abdominal (coelomic) fluid, invert the sedated fish so that the viscera falls away from the ventral abdominal wall, insert a 23- to 25-gauge 1-in needle attached to a syringe under the ventral scales into the posterior abdominal cavity, and gently aspirate with slight negative pressure. Stained and unstained preparations of these samples can often provide rapid, presumptive, or definitive diagnostic information. Samples can also be evaluated by standard cytologic, histologic, bacteriologic, viral, or parasitologic techniques.

**Imaging techniques**—Radiography has been used in fisheries sciences to facilitate the nondestructive examination of the size and shape of the vertebrae, fin rays, and other skeletal elements of fish. The use of radiography as a diagnostic tool in aquatic medicine is presently of limited value but can provide an initial evaluation of morphologically abnormal or injured fish. Radiography of a fish is accomplished by lightly sedating the fish with an anesthetic, removing the fish from the anesthetic solution, and positioning the fish on a piece of plexiglass or plastic wrap covering a film cassette containing high-detail film. After the radiographic film is exposed, the fish is immediately returned to a container of fresh aquarium water for recovery. Alternatively, radiography can be performed on fish directly in water by use of a plexiglass restraining box. Although this can decrease the amount of stress on the fish, this technique requires a greater exposure and generally produces a lower quality image. Special imaging techniques, including xeroradiography, ultrasonography, computed tomography, and magnetic resonance imaging, have also been used as diagnostic tools in fish.

**Discussion**

Through the correlation of clinical history, water quality variables, and results of diagnostic testing, an informed plan of action can then be devised to correct either acute or chronic problems in aquatic animals.
As with most nondomestic pets, client communication is essential and may take longer than the typical domestic animal appointment; however, the rewards of offering these clinical services to owners of aquatic animals can be highly satisfying.

References