AQUACULTURE INFORMATION SERIES NO. 6

METHODS OF TREATING SYSTEMIC BACTERIAL DISEASES

INTRODUCTION

This presentation consists of describing a sound medical approach to preventing and/or controlling systemic bacterial diseases in confined fish populations. With the diagnosis that an episode of systemic bacterial disease is occurring, the process begins with an antibiogram, which consists of determining qualitatively and quantitatively the sensitivity of the bacterial pathogen to a spectrum of antibacterials. The nature and severity of the episode dictate the method of antibacterial administration, of which three major approaches will be described. The final step in the process is to identify the reservoir(s) of infection and remove them and/or their influence from the affected system.

ANTIBIOGRAM

There are many methods of assessing the antibacterial sensitivity spectrum of a bacterial pathogen. The one which seems to be the most time-saving and very reliable begins with the preparation of standardized sensitivity discs impregnated with standardized concentrations of the antibacterials permitted to be used for food and game fish. In America the FDA-approved antibacterials for administration to foodfish would be oxytetracycline, sulfamerazine, and Romet-30. This method can be done under field conditions without having special equipment such as an incubator and a wide variety of media.

Many investigators use commercially prepared discs impregnated with several antibacterials used in human and veterinary medicine. This practice should be avoided for obvious reasons unless a permit (INAD) for their use has been obtained from the regulatory authority (FDA).

There are several methods by which the minimal inhibitory concentration (MIC) of an antibacterial may be determined (Bell et al, 1969; Thornsberry et al, 1977; Simon and Yin, 1970; Barry, 1976). The most frequently employed are the agar dilution technique and the broth dilution technique, in which serial two-fold dilutions of the candidate antibacterial are incorporated into either and agar or broth medium. Both are more suited to laboratory settings than to field settings. The third technique, a modification of the qualitative disc technique, is suited for field application but lacks the sensitivity of the broth and agar techniques.

Of the three foregoing methods to determine the MIC of an antibacterial for a particular bacterial fish pathogen, the most sensitive is the agar dilution method. However, it is not suited for field work because of the necessity for specialized equipment. Also, the method is suited for determining the MIC of up to 12 bacterial isolates. Using this method to determine the MIC against just one pathogen can be quite expensive.

The broth dilution method is more suited for utilization outside the laboratory because prepared tubes can be carried into the field setting and it is suited for one bacterial isolate. The main difficulty with this method is the determination of the end-point, which is based upon turbidity created by the bacterial cells.

The disc dilution method is simple, easy to implement and is quite time-saving. Its main drawback is its inherent insensitivity. That not withstanding, it is suited for field conditions and does afford the investigator the opportunity to establish the treatment regimen validly on the spot.

The rationale for determining the MIC is quite straightforward. First, based upon the qualitative antibacterial sensitivity test, the candidate antibacterial does inhibit the growth of the pathogen(s) involved, the level or degree of the sensitivity must be known for several possible reasons. First, the therapeutic level of antibacterial to be administered must be determined. Second, any changes in sensitivity can be identified and adjustments in the treatment regimen may be made accordingly. Third, there are cases where the antibacterial sensitivity and/or the MIC data can be of great assistance in identifying and/or confirming the reservoir of infection.

ANTIBACTERIAL TREATMENT REGIMENS

There are three methods of administering antibacterials to populations of fish in the farm setting. The most frequently used method of chemotherapy is the oral administration in which the antibacterial is incorporated into or onto the feed. The second method is the administration of the antibacterial via the water either as a bath, a flush or as an immersion. The third method is the administration of the antibacterial via parenteral injection. Each method has its unique benefits and requirements for administration.

Implicit in making the choice of which method to use are the following: (1) size of the population to be treated; (2) age and/or size of the fish in the population; (3) the cost and/or availability of the antibacterial; (4) the amount of antibacterial required to effect therapy; (5) the type(s) of rearing units; i.e., raceways, troughs, ponds, or net pens; (6) the inherent limiting qualities of the technique itself; (7) the pharmacokinetic and toxicological properties of the antibacterial.

A. Oral administration:

Antibacterials are administered to populations of fish either incorporated into the feed before pelletization or as a top-dressing of the feed on site. In addition, there are instances where both methods of feeding have been used.

In general, of the methods of antibacterial therapy, the oral approach is by far the most simple. Its main shortcoming is that clinically ill fish and those soon to become so do not eat, thus they do not acquire the benefits of the antibacterial. Another shortcoming is that in some cases the level of antibacterial is insufficient to affect therapy. This is one of the reasons for determining the MIC before the fact.

In the feeding of antibacterials, the first considerations are the amount of antibacterial required and the duration of the feeding regimen. The recommended daily regimens are:

Oxytetracycline (Terramycin) 2.5-3.75 g/100 lb fish for 10 days

Sulfamerazine - 10 g/100 lb fish for 14 days

Romet-30 - 50 mg/kg fish for 5 days

At the end of the feeding regimen, the following periods of withdrawal must occur before the fish are process or released to the wild:

Oxytetracycline - 21 days

Sulfamerazine - 21 days

Romet-30 - 42 days

A modification of the daily feeding regimens is becoming widely used. During the first three days of chemotherapy the fish are fed twice the recommended level of antibacterial and during the subsequent four days they are fed at the recommended level. This practice is similar to that practiced in human and veterinary medicine. The rational is (1) to provide high blood levels of the antibacterial as quickly as possible thereby precluding - or at least reducing - the potential for inducing antibacterial resistance and (2) to prevent those fish in the incubatory phase from becoming clinically ill.

To accomplish twice the recommended levels of antibacterial in the feed, the feed can be top-dressed with the additional antibacterial on the facility. For most situations, mixing the required amount of antibacterial in vegetable oil at the rate of 500 ml oil per kg feed will suffice. The mixing of large quantities can be done using a clean, small concrete mixer.

The oral administration of antibacterials can also be used to prevent clinical episodes of systemic bacterial diseases in salmonids. The cases where such practices have been beneficial are those in which an episode occurs within days following some physical handling of the fish. For example, the processes of grading for size or the transfer of fish from one rearing unit to another can be followed within 3-5 days by an episode of furunculosis or enteric redmouth disease. Some fish farmers accept this as one of the risks of fish farming. That as it may be, but such episodes can be prevented in many cases.

The rationale for the approach is based upon the following physiological considerations. During and for a short time following the physical handling of the fish there is cessation of intestinal motility. Since digestion in salmonids is primarily enzymatic rather than microbial, there is considerable heat generated in the digestion process. In a static gut the heat can be sufficient to literally "cook" the mucosa. Within a matter of hours following the physical activity there is a resumption of gut motility - often there is a short period of hypermotility. The evidence for this is an increased number of fecal casts on the bottom of the pond within 18-24 hours. Attendant to the sloughing of the mucosal lining there are small hemorrhages which serve as portals of entry for bacteria residing in the gut lumen. Thus, if a potential pathogen were lurking in the gut lumen and the hemorrhages permit access to the underlying lamina propria. In many cases, a clinical episode will begin within 3-5 days. The following procedure is designed to preclude this occurrence.

First, the history of such induced episodes should be documented. Attempts should be made to identify as many of the antecedent causal factors as possible. The bacterial pathogen should be demonstrable in the gut environment and should be isolated to determine the antibacterial sensitivity spectrum and the MIC. Beginning at a point 7-10 days prior to the scheduled physical activity the fish are fed twice the recommended therapeutic level of the appropriate antibacterial for three days, which is followed by 2-3 days of

regular feed. The population at risk is held without feed for 2 days prior to the handling.

The foregoing approach probably does nothing for the cessation of gut motility or its rebound activity following the handling. It does, however, provide sufficient blood levels of antibacterial before the "invasion". The starvation period reduces the amount of mucosal damage by reducing the amount of ingesta.

B. Water administration:

Antibacterials to control external bacterial infections are administered as water-borne chemotherapeutants via several methods: (1) flush; (2) static bath; (3) continuous drip; (4) dip; (5) dip with uptake enhancers.

In all cases of water administration of any therapeutant there are some rather rigid rules to consider (cf. Aquaculture Information Series No. 5). First, the toxicity of the chemical for the fish must be determined. In this process it is suggested that fish from the population to be treated be evaluated. After the exposure period the fish should be examined for sign of toxicity and for the presence/absence of the target pathogen. The test doses should be based upon in vitro data of activity against the target pathogen. In many cases, to release a used therapeutant into the facility discharge is not permitted.

With respect to using water-borne antibacterials to prevent systemic bacterial diseases, the most common application is in the water hardening of fertilized salmonid ova to preclude the vertical transmission of Renibacterium salmoninarum. In this case, fertilized ova are water hardened for 60 minutes in 4 mg/l erythromycin phosphate. For this technique to be optimally effective the female from whom the ova were taken should have received 1 or 2 parenteral injections of the appropriate antibacterial during the maturation period. The process and rationale for this will be presented later.

With respect to reduction of intestinal carriers of bacterial pathogens, the technique most effective at this time is the dip. The process begins with an assessment of the prevalence of intestinal carriers of potential systemic bacterial pathogens. The technique employs immunofluorescent assay of smears of intestinal swabbings of anesthetized fish. The organism is isolated for an antibiogram and MIC values. The selected antibacterial is evaluated for toxicity for the fish in the application environment, which includes the antibacterial in an aqueous solution with an added surfactant; i.e., Turgitol or sodium laurel sulfate, or 3-5% NaCl to enhance uptake (Markwardt and Klontz, 1989). Following the treatment, the prevalence of the target pathogen is again determined. This technique should be applied during the autumn months when the risk of reinfection is minimal.

C. Parenteral administration:

The parenteral administration, via injection, of antibacterials to salmonids is practical in the cases of adult fish. It's major purpose is two-fold: (1) To prevent mortalities during the pre-spawning holding period, and (2) To reduce the potential of vertical transmission of a bacterial pathogen; i.e,. Renibacterium salmoninarum.

The two major routes of parenteral administration are the intraperitoneal (IP) and the subcutaneous (SC). The intramuscular (IM) route

is not recommended because of its potential to create sterile abscesses. This result is due, in main, to the paucity of blood supply to the white muscles. The IP route is very conveniently executed by inserting the needle craniad into the depression between the insertion of the ventral fins Care must be taken to avoid depositing the antibacterial in the intestinal lumen or other visceral organs. The SC route is also conveniently accomplished but not as easily as the IP route. In this case the needle is inserted craniad slightly lateral to the anterior insertion of the dorsal fin in the depression between the muscularis superficialis dorsalis and epaxial muscles. The needle is angled so that it lies within the subcuticular space throughout its length. With the deposition of the inoculum the needle is withdrawn rapidly and the fish returned to the water.

As with other non-oral route of administration of antibacterials, the acute toxicity levels for the fish must be determined. Fortunately, the majority of antibacterials currently administered in these fashions have MIC's which are 20->100X less than toxic does.

BIBLIOGRAPHY AND SUGGESTED READINGS

Austin, B. 1985. Chemotherapy of bacterial fish diseases. In Fish and Shellfish Pathology, A. E. Ellis (Ed), p. 19-26, Academic

Press.

Austin, B. and D.A. Austin. 1987. Bacterial Fish Pathogens. Ellis Harwood Series in Aquaculture and Fisheries Support. Halstead Press.

Markwardt, N. M. and G. W. Klontz. 1989. A method to eliminate the asymptomatic carrier state of Aeromonas salmonicida in salmonids. J. Fish Dis. 12:317-322

Piper, R.G., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler and J.R. Leonard. 1982. Fish Hatchery M

anagement. Dept.Interior,U.S.FishandWildlifeService.

Schnick, R.A., F.P. Meyer, D.L. Gray. 1986. A guide to approved chemicals in fish production and fishery resource management. Cooperative Extension Service, University of Arkansas and U.S. Fish and Wildlife Service. MP 241.

Simon, H.J. and E.J. Yin. 1970. Microbiossay of antimicrobial agents. Appl. Micro. 19(4): 573-579.

Thornsberry, C., T.L. Gavan and E.H. Gerlach. 1977. New developments in antimicrobial agents susceptibility testing. In Cumulativ

eTechniquesandProceduresinClinicalMicrobiology,J.C.Sherri

s(ed.)p1-13,Am.Soc.Microbiol.

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