Bacterial Granulomatous Disease in Fish

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Purpose:

- Provide an overview
- Discuss major differentials and their presentation
- Useful methods for diagnosis and interpretation
- Discussion will mainly surround *Mycobacteria* sp., *Nocardia* sp., and *Francisella* sp.
Mycobacteria

- Mycobacteria- affects a wide range of marine and freshwater species-probably all are susceptible
- Extremely common- *M. marinum* is a frequent isolate
- Many species being implicated as methodology improves
- Gram positive, acid alcohol fast, forms pleomorphic rods, filaments, or coccoid forms
- Lowenstein-Jensen or Middlebrook 7H10 w/ OADC (oleic acid albumin dextrose catalase) media. *M. marinum, M. fortuitum, Nocardia* spp. will grow on blood agar at 23-28C if kept moist
- *M. marinum* often grows in 7-14 days, other species take 2-3 months
- Cultures are not always obtained from diseased fish
- PCR analysis: speciation requires 16S rRNA + housekeeping genes
Mycobacteriosis

- Character of disease: Any variety of external lesions—ulcers, exophthalmia, emaciation, or none
- Internal – prototypical granulomas (aggregations of macrophages forming discrete nodules), particularly in the spleen, kidney, and liver. Appear as grey-white distinct to coalescing foci
Mycobacteriosis

- Microscopically, granulomas begin as groups of macrophages, with/without progression to central necrosis and gross caseation.
- With chronicity the fibrous capsule is pronounced.
- Granulomas can also be found commonly in cases of fungal (*Ichthyophonus* sp.) and parasitic infection.
- In acute cases, no gross lesions may be seen.
Granuloma

- Early stages- macrophages form discrete aggregates
Granuloma
Granuloma
Granuloma
Mycobacterial acid fastness

- The capacity of mycobacteria to resist decolorization with acid alcohol
- Potential pitfall
  - Not specific; *Nocardia* spp., *Rhodococcus* spp.
  - Not sensitive; less than 1% of bacteria may retain stain
- A function of; intact bacterial cell walls (trapped fuchsin), mycolic acids (varies by species, growth conditions), stain methodology, fixation method (formaldehyde is detrimental), growth stage of bacteria

Acid fastness in a mycobacterial sense is to be distinguished from general acid fastness- some maintain that only mycobacteria resist decolorization w/ 3% acid alcohol. Modified stains use mineral acid.
Mycobacterial acid fastness

- “Ziehl-Neelsen” often used- not a standardized stain, a “catch-all” for many recipes and variations. Methodology should be reported in articles.
- Lack of acid fast organisms doesn’t rule out mycobacteria – not all mycobacteria have thick cell walls or any cell walls.
- Fixation w/ 60-80% ethanol or 10% TCA may improve acid fast staining in sections.
- Additional evidence (culture) needed for diagnosis.
- Isolation of mycobacteria, the presence of lesions, and acid fast bacteria in lesions may not be well correlated.
- Decalcification may severely hamper staining.
- Periodic acid (oxidizing agent) sometimes advised in order to increase staining- such treatment = no longer acid fast in a mycobacterial sense.
Acid fast *Francisella*

- 20 minute treatment w/ 10% periodic acid can turn *Francisella* sp. acid fast w/ ZN stain
- Not an appropriate diagnostic approach alone
Nocardia

- *Nocardia*: affects fresh and saltwater species, less frequent than Mycobacteriosis
- Gram positive, weakly acid fast, branching rods, up to 3 weeks to grow in media (blood agar, TSA, BHI, LJ) at 25C
- Branching aerial hyphae on culture dishes
- *N. asteroides*, *N. seriolae*, *N. salmonicida*
- Clinical signs similar to mycobacteriosis, sporadic
Nocardia

- Often stated as acid fast, depend heavily on the method applied. Modified stains using mineral acids rather than acid alcohol may be more effective.
- 3% acid alcohol is the standard ZN decolorizer – *Nocardia* won’t retain stain. If 1% acid alcohol is used (some commercial ZN stains), *Nocardia* will retain the stain.
Nocardia, periodic acid, ZN stain
Francisella noatunensis, *F. asiatica*

- An emerging pathogen - an increasing list of affected species, even mollusks. Cod, tilapia, salmon, cichlids, hybrid striped bass
- Gram negative coccobacillus, highly virulent
- Won’t grow in simple media, often difficult to isolate
- Slow growing, contamination a problem
- Cysteine heart agar + bovine hemoglobin + ampicillin + polymyxin B, 15-30 C.
- Often confused with Piscirickettsia like organisms (PLO) which are problematic in Chilean salmon.
Francisellosis

- Severe granulomatous disease, particularly in the head kidney, spleen, and liver.
Francisellosis

- Granulomatous branchitis, necrotizing myositis
Francisellosis

- In tilapia, spleens may be affected such that they become pale tan and spongy due to cystic cavitation
Franciselllosis
Francisellosis
Other reported causes of granulomatous disease in fish

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