Serology in finfish

for diagnosis, surveillance, and research: a systematic review

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Outline

- History
- The fish immune response
- Systematic review
- Results
- Discussion

Background

What is a serological test?

- Serological tests detect antibodies, or antibody-like substances, primarily in serum, but also in other bodily fluids: mucous, milk, semen, and saliva
- Assay detects the reaction that occurs when an antibody binds to an antigen

Types of serological tests

- flocculation tests, e.g. complement-fixation test (CFT)
- neutralisation tests, e.g. serum or virus neutralization (SNT/VN)
- haemagglutation (AGG)
- enzyme-linked immunosorbent assay (ELISA)
- direct or indirect immunofluorescent antibody test (FAT, IFAT)
- precipitation tests, e.g. agar gel immunodiffusion (AGID)

Background

"Because of the insufficient development of serological methodology, the **detection of antibodies to pathogens in fish has not thus far been accepted as a routine method** for assessing the health status of fish populations" (OIE Manual 2012).

"An **assay for antibodies would not be an acceptable** test in a fish health inspection program for *Renibacterium salmoninarum"* and "**serological methods cannot be recommended** alone in fish health screening programs"

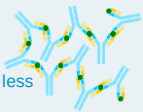
Background

The OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2015a) describes few serological tests.

Recommendation – OIE global aquatic animal health conference

Request the Aquatic Animal Health Standards Commission to consider the development of recommendations for the use of sero-surveillance for fish and for the concept of disease freedom at supranational level.

The fish humoral response



- Finfish antibodies limited to a dominant IgM and a less abundant IgT (also known as IgZ)
- Fish lack antibody-class switching, & the affinity of specific antibodies does not increase following repeated immunization
- If an immune response develops against a pathogen's antigens it is highly specific
- Temperature, other environmental and host factors (e.g. genetics and age) influence the immunological response

Material and methods

Material and methods

- Systematic review following guidelines in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement
- Searches on the Web of Science using the following search terms with both British and American spellings

PRISMA

THOMSON REUTERS
Web of Science

Search term

[disease OR pathogen] **AND**
[(antibody OR antibodies OR immunoglobulin) **OR**
(serology OR serological OR serodiagnosis)]

For example, the search terms for VHSV included:

["Viral haemorrhagic septicaemia" or "Viral hemorrhagic septicemia" or "VHSV" or "VHS"] and [(antibodies or antibody or immunoglobulin) or (serology or serological or serodiagnosis)]

Validation

- Search results for two of the pathogens in this study were compared between the Web of Science and CABI databases
- Additional titles were identified manually through citations found in articles identified in the initial search

Pathogen selection

- To include common pathogens of significance in the most commonly exploited fish species
- Included all finfish diseases in the 2015 OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2015a) and diseases listed in the compendium of fish diseases (Woo et al. 2011)
- Additional pathogens added based on the expertise of the authors

Screening

- Two stage : by **title** and then **abstract**
- Repetitions and unrelated titles (describing species other than fish and pathogens outside the scope of the review) were excluded from the analysis
- All titles in languages other than English, or published in conference proceedings or grey literature, were excluded.
- Screening done by three reviewers and a confirmatory analysis by a fourth reviewer

Categorisation

- **Infectious agent**
- **Year of publication**
- **Host species**
- **Subject of the publication (test development, test evaluation or test application)**
- **Type of serological test/tests**
- **Purpose of test/tests (surveillance, post-vaccination monitoring or immune response)**
- **Inclusion of positive and negative control sera**
- **Reaction specificity analysis (inclusion of internal controls for the confirmation of the specificity of the reaction)**

Further analysis specific to articles involved in test development or test evaluation

- **Optimization**
- **Accuracy analysis (reporting of sensitivity and specificity, both analytical or diagnostic)**
- **Statistical analysis**

Results

Results

- 841 papers from initial search
- 549 after exclusions for repetitions
- Additional 19 papers found in other searches
- 204 papers met eligibility criteria based on screening title & abstract
- 184 after further screening of full-text (of which 7 were unobtainable)
- 176 papers included in the analysis

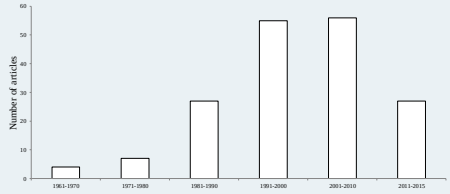
Categorisation

Pathogen
- 53% bacteria
  - 7% Aeromonas
- 36% viruses
- 10% parasites
- 1% fungi / oomycetes

Host
- 58% salmonidae
  - *O mykiss, S salar*
- Channel catfish
- Carp

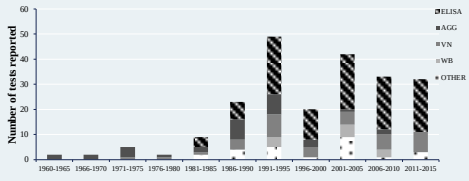
All Serological tests found for all OIE listed diseases except OMV and G. salaris

Temporal trends – number of articles



Assays

- Agglutination tests has been replaced by ELISA and VN



Purpose

- 35% development and / or evaluation to tests
  - 15 reported procedures to optimise tests
  - 7 reported diagnostic test sensitivity and specificity
- 65% application of tests
  - 35% research into immune response
  - 33% assessment of post-vaccination immune response
  - 24% disease surveillance
    - Only 2 articles reported use of to demonstrated disease freedom

Discussion

Advantages of serological tests

- Serology has advantages over challenge-survival in vaccine testing
- Non-lethal
- Cost-effective and sensitive at population level
  - Antibodies persist longer and more easily detectable than the infectious agent
  - Detection of subclinical / latent state
- Effective, cheap tests supports aquatic health management by making surveillance to demonstrate freedom easier

Why are there so few studies reporting the use of serology to demonstrate freedom?

Limitations

- Lack of full validation
  - Lack of control of non-specific reactions
  - Internal controls should be used to assess non-specific binding for ELISAs
- Few estimates of test sensitivity and specificity
  - Bayesian latent class models should be used to compared tests with imperfect reference standards
  - Evaluation of serological tests against direct detection methods can be biased (and dependent on stage of infection)
  - Timeline for antibody production during and after infection needed

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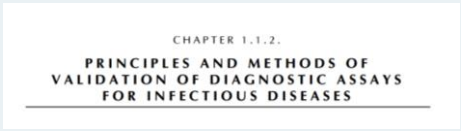
Manual of Diagnostic Tests for Aquatic Animals


CHAPTER 1.1.2.

PRINCIPLES AND METHODS OF VALIDATION OF DIAGNOSTIC ASSAYS FOR INFECTIOUS DISEASES

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Conclusion

- Serological tests are complementary to direct detection methods
- Wider use of serological tests will support aquatic animal health management
- Increased application requires investment in
  - Evaluation of test performance
  - Quality control
  - Baseline serological response studies

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