DIAGNOSTIC TEST VALIDATION: EPIDEMIOLOGICAL APPROACHES

Larry Hammell
Professor, Dept of Health Management
Atlantic Veterinary College
University of Prince Edward Island
Charlottetown, Canada

Edgar Brun
Director
Division of Epidemiology
Norwegian Veterinary Institute
Oslo, Norway

Co-Directors
OIE Collaborating Centre:
Epidemiology & Risk Assessment of Aquatic Animal Diseases
Guiding principle

- Assessment of detection performance should mimic the field conditions
Surveillance for declaration of freedom

- Active surveillance
  - Selection of farm / units
  - Selection of fish
  - Selection of tissues
  - Selection of tests

- Focus now on tests
  - Assessment under the same conditions of use in surveillance
    - Includes fish handling, tissue collection methods (aseptic but field conditions), submission in groups that may affect contamination
Primary Considerations

- Diagnostic performance assessed under conditions of intended use

Samples from lab infections

If using for farm surveillance, will lab exposure and infection patterns produce different characteristics?

Samples from sick individuals

Meaningful for use as confirmation;
But surveillance use often in apparently healthy animals
Surveillance in apparently healthy animals

Confirmation in sick animals

cage 21 (ISA outbreak)
Surveillance

Apparently Healthy: poor Se

Mortalities (with ISA): improved Se

Example Mortality Pattern
Surveillance Frequency

- Depends on health visits for examining moribund individuals
  - Key to early detection is investigating increased mortalities / moribund
  - Reporting of mortality records / patterns crucial
Assessment of Diagnostic Se / Sp

- Estimation in absence of known status
- Requires access to different populations with different pathogen prevalence
  - Latent class analysis
    - At least 2 populations, 2 tests (unrelated)
    - Prefer more
    - Greater sample size within populations is best (n>100?)
Performance for different genotypes

- Testing using one genotype may not represent other genotypes
  - Needs work with new genotypes
  - Needs constant re-assessment as new genotypes discovered
Performance for new diagnostic tests

- As new diagnostic tests are developed or refined, need assessments using natural exposure tissue samples
  - Different prevalence populations
  - Different diagnostic tests
Sampling from different prevalence populations

- Capacity for rapid mobilization of resources to sample from outbreaks and neighbors
  - Gives high prev - sick animals
  - Moderate prev - neighbor animals: exposed animals not showing clinical signs in same group
  - Low prev - similar timing but in neighbor site
  - Zero (or near-zero) prev - population not known to be exposed
“At least 2 tests & 2 populations of different prevalence level”

Detection Tests

Extracted RNA

RT-PCR (segment 8)

qRT-PCR (probe)

VI (SHK & ASK)

IFAT

Commercial Kit

Slide credit: Charles Caraguel
Sampling from different prevalence populations

- Capacity for rapid mobilization of resources to sample from outbreaks and neighbors
  - Multiple tissue samples per animal
    - E.g. 6 RNALater tubes of kidney to send to different labs
  - Labelling ready for sample collection
  - Multiple technical staff needed, short notice

- Location, timing, readiness ........ funding
Sampling considerations

- Samples need to be treated in the same manner
  - Similar methods to collect sample
  - Aseptic methods – as in routine sample collection
    - Field samples in routine, then field samples in collection methods
  - Similar storage methods and duration
- -80 storage facilitates process
Sampling considerations

- Randomization of samples to lab
  - Pathogen unevenly distributed within tissue has equivalent probability of going to any lab
  - Sample collection fit to routine surveillance
    - So lab does not receive sample not routinely submitted
  - Need possible lab contamination as during routine submissions
    - Random order of pos & neg samples
Sampling considerations

- **Blinding of samples source**
  - Mimic routine surveillance when no expectations
    - Should not be able to identify samples coming from outbreaks
  - May have positive in one sample and negatives in other samples
    - Contamination possible – just like during routine surveillance
Test interpretation

- Getting zero prevalence samples is dangerous for the “free” area
  - Interpretation of false positives
  - Don’t contribute samples if results linked back to region status
Conclusion

- So who is actually collecting field samples from outbreaks and non-outbreaks??
- International community needs a shared archive of appropriate tissue samples from different prevalence populations
  - There is a need for concerted effort to get natural exposure samples and share across labs in world (not just within region)
Summary Points

1. **Diagnostic Sensitivity and Diagnostic Specificity** inform decision making
   - But rarely available
2. **Rigorous epidemiological assessment** of Diagnostic Se / Sp & repeatability are needed
3. **Latent Class Analyses** can be used to estimate test performance characteristics using production cases
4. **Natural outbreaks** represent most realistic exposure / infection pattern
5. **Field collection from different prevalence populations** is on-going need
6. **Sharing of field collected samples** across labs (and regions) important to assessing laboratory / test performance
7. **Sharing of experience** in outbreaks should be incorporated into our expertise building
8. Need **pre-arranged funding and capacity** for doing field research in unpredictable, emergency situations (i.e. depopulation)