EARLY DISEASE DETECTION

SAMPLING AND DIAGNOSTIC TESTS IN APPARENTLY HEALTHY POPULATIONS

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Traditional approaches to Diagnosis & Prognosis

- How do we detect abnormalities?
- Experience / training, gut reaction & intuition
- Some wise individuals just know from experience (and ability to convince others that they are correct)
- How do we translate diagnosis in sick animals to surveillance in apparently healthy animals?

Why use apparently healthy animals

- Preventive medicine depends on early detection
- Early detection cannot wait for mortalities to start increasing
- So using healthy population, maybe healthy individuals to satisfy need for earlier detection

Sampling principles

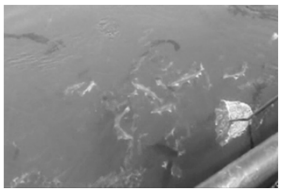
- Random sampling (= probability sampling)
  - Simple random
  - Systematic random
  - Multiple staged, random sampling
    - E.g. area / farm / pond
- Biased sampling
  - Convenience
  - Risk-based = syndromic

Random Sampling

- Systematic random sampling is most common form of random
  - Want 100 samples from 10,000 fish unit
  - Then take every 100th fish, starting with random in first 1-100
    - E.g. 27, 127, 227,,, 10027, 10127
- Can apply to different levels of concern

Biased sampling

- Convenience samples
  - Feeding and catching fish with dip net



Biased sampling

- Risk-based (i.e. syndromic) samples
  - Moribund with specific external characteristics known to be more common for disease of interest

Disease detection

- Diagnostic tests are imperfect
  - Particularly when attempting to detect asymptomatic individuals
- New cultured species *will* have new pathogens identified

Diagnostic tests in aquatic situations

- Disease is frequently assumed to be absent in facilities that lack obvious clinical disease
- Subclinical and carrier states are a major consequence for non-stressed animals
- Diagnostic tests cannot detect many non-clinical disease states
  - Many new diseases have no tests except general pathology to identify suspicious agents or host responses

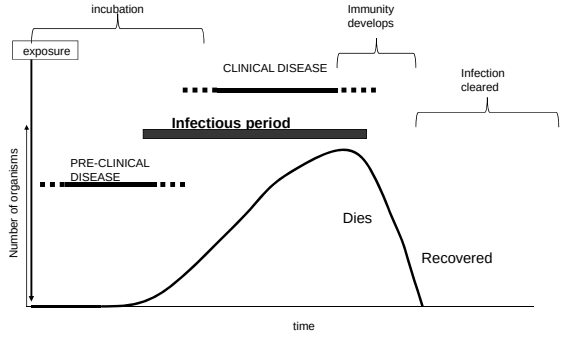
Comment about working with new species

- New diseases (i.e. new host-pathogen interactions) are most likely to occur when working with new species AND working with several new species at same time
- Not likely to recognize the event when it first happens

Virus X example

- Diagnostic tests (ELISA)
  - Correctly detect 96% of positive cases (sensitivity)
  - Correctly detect 98% of negative cases (specificity)
- So why do we approach a positive test done in a healthy animal much differently than a positive test in a clinically sick case?

Pathogen replication in host



Clinical signs

Disease Surveillance

- Sample from 25,000 fish cage
  - From 10-20 cages per site
  - From 75-90 sites
- Test with which test
  - Virus isolation - 3-21 days, $100/test
  - Indirect florescent antibody test (IFAT) – 1 day, $15/test
  - Enzyme Linked Immunosorbent Assay (ELISA) – 1 day, $20/test
  - RT-PCR – 1 day, $45/test

Background

- Sensitivity (Se): Probability that an infected fish will test positive
- Example: Se = 62%

	D+	D-
T+	**62**	
T-	38	
	100 fish	

Background

- Specificity (Sp): Probability that an uninfected fish will test negative
- Example: Sp = 98%

	D+	D-
T+		2
T-		**98**
		100 fish

Background

	D+	D-	
T+	62	2	64 postive tests
T-	38	**98**	136 negative tests
	100 fish	100 fish	200 total tests

Biasing samples can be good

- We routinely bias our samples toward detection
  - By looking for individuals that have characteristics common in the diseased population
    - Smaller individuals (compared to cohorts)
    - Off-feed or altered swimming behaviour
    - Slow swimmers
    - Fish with lesions

Matter of pre-test probabilities

Apparently healthy population

Sens = 0.96

Spec = 0.98

True prevalence = 0.02

	Disease+	Disease -	
Test +	192	196	388
Test -	8	9604	9612
	200	**9800**	**10,000**

388 individuals test positive, what is the probability that any single individual is truly positive?

Proportion of true positives that test positive = 0.96 * 200 = 192

Proportion of true negatives that test negative = 0.98 * 9800 = 9604

Matter of pre-test probabilities

Apparently healthy population

Sens = 0.96

Spec = 0.98

True prevalence = 0.02

	Disease+	Disease -	
Test +	192	196	388

388 apparently healthy individuals test positive, what is the probability that any single individual is truly positive?

192 / 388 = 0.50 or 50%

Matter of pre-test probabilities

Clinically sick population

Sens = 0.96

Spec = 0.98

True prevalence = 0.20

	Disease+	Disease -	
Test +	1920	160	2080
Test -	80	7804	7920
	2000	8000	10,000

2080 individuals test positive, what is the probability that any single individual is truly positive?

Matter of pre-test probabilities

Clinically sick population

Sens = 0.96

Spec = 0.98

True prevalence = 0.20

	Disease+	Disease -	
Test +	1920	160	2080

2080 sick individuals test positive, what is the probability that any single individual is truly positive?

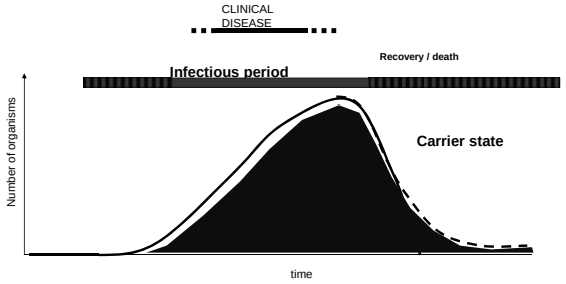
1920 / 2080 = 0.92 or 92%

- Experienced clinicians are good at increasing the prevalence of a condition through identification of clinical signs (physical exam) and history
  - diagnostic tests then have a higher probability of being correct
  - Note: physical exam for clinical signs can be assessed for sensitivity and specificity because they are subjective "diagnostic tests"

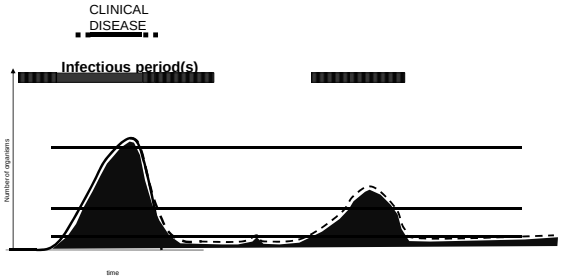
Pathogens: The Host Perspective

- Clinical disease
  - <u>*Externally obvious abnormality*</u> usually leading to decreased probability of surviving or reproducing
    - Direct result of abnormality or indirect (e.g. increased predation)
- Subclinical disease
  - <u>*Abnormality is not externally obvious*</u> (behaviour or lesions absent), but change in probabilities likely
  - Usually reflects our reliance on diagnostic tests for agent or host response to diagnose

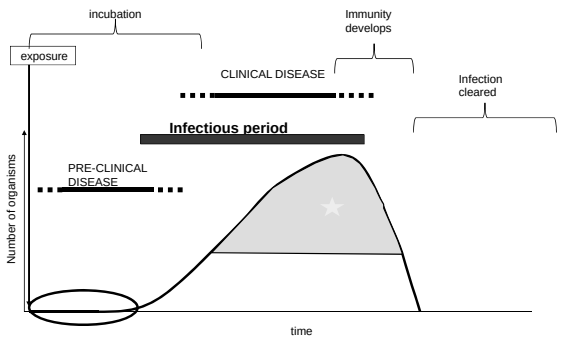
Pathogen replication in host



Diagnostic tests



Surveillance results best applied to animals before they get sick



Detection is affected by many factors

- Disease level in the individual tested
  - Clinical disease is easier to detect
  - Surveillance of 'apparently healthy' individuals is more difficult
- Disease level in the population
  - Which animals are sampled

Depopulation of cage: Does it prevent virus exposure for other cages at site?

Apparent prevalence of different populations (HPR_{ALL}) of Atlantic salmon in New Brunswick farms (2001).

Population	Apparent prevalence (95%CI)	
A^{α}	0.940 (0.887, 0.993)	moribund fish, outbreak cage
B^{β}	0.406 (0.279, 0.533)	healthy fish, outbreak cage
C^{β}	0.286 (0.204, 0.368)	healthy fish, non-outbreak cage, outbreak site
D^{γ}	0.084 (0.009, 0.160)	healthy fish, non-outbreak cage, non-outbreak neighbor site
E^{γ}	0.080 (0.004, 0.156)	healthy fish, non-outbreak cage, distant site

McClure, Hammell, Dohoo, Nerette, Hawkins. 2004. *J Fish Dis 27: 375-383.*

Diagnostic test performance

- What happens when an individual is sick with a virus?
  - When does infection actually occur?
  - When will clinical signs be evident?
  - When does a diagnostic test work best?

Conclusion

- Diagnostic samples must occur in surveillance program
  - Collecting evidence to change our decisions about what is positive and what is negative when fish are sampled *prior* to actual mortality spikes
- Depopulation may be only control measure
  - Costs are high so don't want to decide too early
  - Allowing positive cases to continue can cause entire site and area to continue positive (cost much more!)