

Diagnosing *clinical disease* and *subclinical infections* in a vaccinated population

Pre-AASV BI Health Seminar
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There is sometimes translational or semantic confusion in how veterinarians view infection versus disease. Assumptions and definitions for this discussion follow:

Infection with endemic agents (e.g. PCV2, *Mycoplasma hyopneumoniae* -Mh) is a common event. Current or previous infection can be measured with great sensitivity (e.g. PCR, serum antibody). Infection is not the same as clinical disease.

Disease is abnormal structure or function.

Clinical disease is when disease is recognized by symptoms, lesions, and/or some measurable functional deficit. Disease *in a population* is recognized when one or more individuals have clinical disease. Simply put, clinical disease is when there is some objective observation or measurement that establishes abnormal structure or function of one or more individuals.

Subclinical infection with endemic flora is common. Infection results in no discerned measurement or observation demonstrating deviation from normal structure or function.

Subclinical disease is somewhat an oxymoron. Once a clinical effect (e.g. clinical disease) is recognized, it is no longer subclinical. The rub is that there may be effects of infection that are unrecognized or unknown until serendipity potentiates recognition. How is the concept of the vernacular "subclinical disease" integrated into decisions on farms? Perhaps the concept should be one of **impact**.

Impact of clinical disease in an individual animal is usually assessed by integrating impact of presence of agent(s), extent of lesions, and/or parameters of function.

Impact of clinical disease in a population of animals (a portion of which has disease) cannot always be quantified simply by extrapolation of a clinical disease diagnosis and impact of individual animals. Additional measures from group performance are required, usually combining biological performance indicators and economic performance indicators.

The impact of subclinical infection is not detected by simple observation. Additional measures from group performance are required, usually combining biological performance indicators and economic performance indicators in a field-trial situation. Detecting "it" does not mean that disease is expressed with a significant impact, nor does detecting a very common "it" mean that "it" is not important. The aim of this discussion is to discuss concepts that may be relevant to sorting this out.

Summary: Diagnosing *clinical disease* and *subclinical infections* in a vaccinated population

1. Diagnosing disease is different than confirming infection. Seroconversion or detection of an endemic agent by a sensitive method is not necessarily a “*disease state*”; it may simply be *infection* status. Diagnosis of disease requires evidence of “deviation from normal”. Quantifying the biological or economic impact of infections may not be evident except by mitigating the insult (e.g. a field trial for the intervention).
2. The impact of *clinical disease* is by definition observable or measurable. The challenge is extrapolating individual animal status (diseased or not) to population status (Is it a significant biological, economic, welfare, etc. problem or not?).
3. Vaccination status does not change the methods for diagnosis of *disease* where the agent is endemic. Vaccination will alter the dynamics of transmission, infection, immunity, ecology, and disease expression. Problem-solving skills and analytical tools using accurate data from disparate sources have more value than thumb-rules, opinions, and generalizations. This is especially true for agents where infection is not prevented and sterilizing immunity is not achieved by vaccination (examples: PCV2 and Mh)
4. The significance of PCVAD or Mh in a vaccinated population is relative to expectations of vaccine efficacy and is confounded by co-infections, disease expression risk factors, and vaccination decisions and execution. Effects of subclinical infection are difficult to diagnose by routine “diagnostic tests”. Analysis of accurate records, thorough monitoring for other agents and contributors, necropsy, slaughter checks, and (especially) vaccine field trials are likely to offer better information.

General Comments

It is fairly easy to assign (diagnose) a *proximate* cause of disease affecting an individual animal. Clinical signs, lesions, and evidence for causation allow one to name a disease with some unspecified level of confidence, but that process does not necessarily exclude other agents or factors in causation. On a herd basis, the goal is to not only identify proximate cause of a few animals, but also to find, assess, and prioritize the components of the *ultimate* causes of disease in the herd. Ultimate cause prioritizes all known components contributing to outcome (the multitude of infectious and noninfectious factors). Ultimate cause(s) may not be immediately obvious, may challenge our dogma, and usually require a system or process change rather than a single nostrum as intervention strategy. (Band-aids for proximate causes have limitations) There is no single test that defines causation on a herd basis. Factual knowledge, experience, and **observation** of husbandry, nutrition, pig flow, environment, diseases, infectious agent ecology, complimented with critical thinking and process improvement tools are the foundations for adding context to the complexities.

Frequently, samples are shipped to a laboratory for “diagnosis”. A diagnosis can be general or specific, depending on the information that is available and the perspective of

the observer. In the example of diagnoses from one pig (below), one diagnosis is likely to be more “important” than the others, yet none alone define the disease present nor the risk factors that contribute to disease expression.

1. **Clinical diagnosis**
 - a. Respiratory disease/pneumonia
2. **Pathologic diagnosis**
 - a. Gross: 20% cranioventral gray firmness of lung with mucopus in larger airways
 - b. Histo: Depending on section examined, there is necrotizing bronchiolitis, suppurative bronchopneumonia, nonsuppurative peribronchiolar cuffs, BALT hyperplasia, and lymphoid depletion.
3. **Disease diagnosis**
 - a. Swine influenza;
 - b. PCVAD;
 - c. enzootic pneumonia (Mh and P. multocida)
4. **Etiologic diagnosis**
 - a. Swine influenza virus, confirmed by PCR;
 - b. *Mycoplasma hyopneumoniae*, confirmed by PCR
 - c. PCV2, confirmed by IHC
 - d. *Pasteurella multocida*, confirmed by isolation

A diagnosis offered by laboratory testing is not a herd diagnosis. Herd diagnosis relies on a knowledgeable veterinarian evaluating all the evidence, including diagnostic laboratory data. The goal of diagnostic investigation is accuracy and clarity in test results or defining an abnormal condition. Veterinarians then integrate diagnostic laboratory results using principles of epidemiology, various “ologies”, preventive medicine, record analysis and all available resources, along with experience, to offer a herd diagnosis. An endemic disease agent (e.g. PCV2, Mh) may have significant impact for the individual affected (individual animal diagnosis) but seemingly little impact on herd performance (herd diagnosis), therefore an individual animal or a single test diagnosis must be carefully interpreted when applied to a herd diagnosis. Veterinarians that focus solely on herd information may miss opportunities to improve performance of individual pigs that contribute to herd productivity. Veterinarians that focus on individual animal afflictions may miss opportunities to improve biological or economic performance on a herd basis. It is challenging to do both individual and population medicine well. Fundamental diagnostic questions that may need to be answered include: “Is it there?”, “Is it important?”, “Is it new to the herd?”, “What else is involved?” Generally, we only find something if we look, and what we find is often only that which is sought. Conveying essential information to the diagnostician is critical if a veterinarian wishes to access a diagnostician’s knowledge and experience. Before requesting diagnostic assistance, practitioners may ponder:

1. What is the diagnostic question that I wish to answer in this case? ***It is critical this be conveyed to a diagnostician (fill out submission forms: communicate!)***

2. Which tests can be used to answer the question?
3. What populations should be sampled? Random or targeted sampling?
4. What type and how many samples are required?
5. How reliable is the test and how are the results reported?
6. How will results be interpreted?
7. How will results be applied to formulation of an intervention strategy? What decisions will be influenced by testing outcomes?

The quality of specimens submitted to a laboratory is directly related to the quality of diagnostic information received. Diagnostic laboratories still receive many submissions where haphazard or inadequate sample collection or preservation compromise quality of results. The **diagnostic sensitivity** of a test is easily compromised by poor sampling, even though the **test performance** (sensitivity / specificity) is as expected. If technicians are used for sampling and submission, their training should be a high priority. Tests can be wrong (sensitivity/specificity) and errors occur but specimen selection/quality is by far the biggest reason for dubious diagnostic results. Diagnostic tests are complicated and expensive. A practitioner should seek to understand the test types, what the tests detect, how the analyte is detected, test limitations, and test interpretation

Table 1: Simplified overview of analytes, tests, and methods

Analyte Type	Test types	Basis for Testing
Visualize agent	Gross exam, microscopy, electron microscopy	you can "see it"
Detect viable agent	bacterial isolation, virus isolation	it grows
Detect Antigen	IHC, agELISA, FAT	uses specific antibody
Detect Nucleic acid	PCR, ISH	uses known nucleic acid sequence
Detect Antibody	CF, IFA, Agglutination, HI, VN	uses known antigen
Detect Chemical/element/toxin	GC, AA, ICPMS	analytical chemistry

Each test has strengths and weaknesses. Communication with a diagnostician via submission form or direct communication is essential for complete and accurate diagnostic workups. Each case has unique nuances; standardized testing strategies are useful for monitoring but can miss unique disease permutations in diagnosing disease. **Context impacts interpretation of results.** Healthy skepticism is awareness for what is unknown or not observed and it always questions assumptions. If diagnostic results don't make sense, then there is likely something not right about the process. Review all steps of the diagnostic investigation, from farm observations to sampling to testing to reporting. Miscommunication and misinterpretation of information is quite common.

Vaccine expectations

For both PCV2 and Mh, vaccination (or therapeutic) does not prevent infection; at least some pigs will be positive for infection. Moreover, no intervention will eliminate all lesions in all animals. In large populations of animals there is a good chance of finding individual animals with infection and with lesions (disease). Vaccination or therapeutic is expected to decrease the number of animals affected and the magnitude and duration of lesions. In most vaccination trials, one expects to find lesions and infections in vaccinates, but to be efficacious, there should be sufficiently fewer of them to warrant the particular intervention.

PCVAD

During an unmitigated epidemic of PCVAD, many pigs are affected in dramatic fashion. The diagnosis of herd impact is easy and made with confidence. The criteria for diagnosis of PCVAD proposed by Sorden (2000):

1. clinical signs characterized by wasting/failure to thrive, with or without dyspnea or icterus;
2. histologic lesions characterized by depletion of lymphoid tissues and/or lymphohistiocytic to granulomatous inflammation in any organ, typically lungs and/or lymphoid tissues; and
3. PCV2 within characteristic lesions, usually demonstrated by IHC.

Vaccination has been overwhelmingly acknowledged as beneficial for PCVAD, yet PCV2 remains an endemic infection. Endemic infections generally permeate the population over time; *not all individuals in a pen, barn, or site are of equal infection or clinical status*. If “something bad” happens as a result of infection AND it is recognized, then there is **clinical disease**. If nothing bad is recognized, then it is said to be a **subclinical infection**. The “something bad” part usually needs to be quantified at the herd level. The ecology of the potpourri of infectious insults in populations assures that not all pigs have the same experience with insults at the same time or same sequence. That individual pigs in a population will be found diseased in vaccinated, endemically-infected herds should not be a surprise. There was and remains considerable variability associated with cases of PCV2-associated disease. There is variation in symptoms; dramatic outbreaks and wasting are rare but variation in growth rate and niggling ill-thrift is often observed. Because “PCV2 tends to go where inflammation is”, there is considerable variation in tissues affected (gut, heart, brain, liver, kidney, lung, lymph nodes, synovium, spinal cord). It seems likely that PCV2 has propensity to take advantage of some immune or inflammatory process induced by other infectious agents that are moving through the population at various rates. Moreover, occasionally, (mild) lesions compatible with PCV2 are detected in vaccinated animals in which no PCV2 antigen is detected by IHC.

Because of the permutations of infectious insults that can occur, diagnosis of disease in herds increasingly requires “complete” diagnostic work-ups. **A “complete work-up” is not an SOP**. Rather, it is a diagnostic plan constructed to answer specific questions related to infection status and disease status in the herd. Monitoring the disease status

for endemic infectious agents likely will include routine necropsy with occasional laboratory verification of assumptions. This, too, is confounded by some endemic agents that persist in individuals and populations (e.g. PRRS, PCV2, Mh, nasopharyngeal bacterial flora) versus those that tend to be transient (e.g. SIV). Establishing a role for disease contribution for sporadic agents or new agents likely will require clinical assessment as well as laboratory submission of tissues and/or serum from various age groups over time.

Epidemiologic principles applied with random sampling using quantitative PCR perhaps could be useful, but test interpretation is fraught with sources of interpretative error, and this process is usually unacceptable because of high cost. Sampling sick pigs with PCR may confirm PCV2 involvement, but the result cannot be extrapolated to the herd. The effect of other infectious processes, known or unknown, likely has some additive or synergistic effect with PCV2.

Probably the key to determining if an endemic infection is clinical disease with herd impact (rather than subclinical infection) is by some measurement of biological or economic significance in the group. Proactive measurements (mortality, morbidity, incidence, prevalence, clinical signs, lesions, growth rates, performance variation, and innumerable diagnostic tests) help us define what we know or suspect. When in doubt, **probably the best way to determine if clinical disease or subclinical infection is truly having a significant biological AND/OR economic impact, is by conducting field trials using a properly designed scientific study.**

The diagnosis of PCVAD in a vaccinated population is the same as in an unvaccinated population. If one or more animals have disease associated with PCV2, then PCVAD is present in the herd. Expectations of vaccine efficacy should be pondered, especially with those agents (e.g. PCV2, Lawsonia) that have significant consequences for individual animals that are not vaccinated or protected. Random thumb-rules (e.g. only 80% of population needs to be vaccinated to achieve control) do not apply to PCV2. The question becomes one of overall impact and expectations. Again, the overall impact is unlikely to be accurately assessed by single animal/sampling protocols. What are some of the confounders to diagnostic processes, interpretation of evidence or thought processes?

Basic assumptions of this diagnostician

1. PCV2 infection of pigs is ubiquitous, irrespective of vaccination status with seroconversion around 10 weeks +/- 6 weeks and substantial variation (exposure dose, timing of infection, susceptibility, and levels of maternal antibody);
2. Vaccine is being used widely because it mitigates clinical disease and lesions (the fact that it works well despite the great variation in how it is selected and applied suggests it has a wide margin of error) but it does not eliminate viral load in the pig and the environment;
3. Humans are innovative to the point of whimsical and will figure out a way to compromise efficacy or blame the wrong input. Perceptions (opinions) can trump

realities (data) in everyday practice. The impact of denial or failed processes are often underestimated;

4. Failure to diagnosis and control confounders (other diseases, risk factors) is blame-shifting;
5. Sufficient, accurate data adds confidence but the wrong data, inadequate data, poorly interpreted data may foster a perception of more confidence in a decision than is warranted (also known as blame-shifting).

Tools for diagnosing PCV2's role in disease in a vaccinated herd include

1. Clinical examinations of pigs by astute clinical veterinarians
 - a. PCV2 often takes advantage of inflammation inflicted by other infectious agents
 - b. PCV2 is a systemic infection, sometimes with vague or subtle signs
2. Analysis of performance records, estimates of variation, root cause analysis for variation
3. Routine post mortems: “% mortality” is a quantitative but not a very accurate diagnosis
 - a. Routine necropsy by trained staff is for monitoring purposes only
 - b. Critical examination, description and gross diagnosis of specific diseases is best performed by a competent veterinarian
4. Quantitative assessments and odds ratios; actually count animals and calculate incidence and prevalence for clinical signs, lesions, perceived abnormalities
5. Routine laboratory submissions sufficiently comprehensive to establish baselines for endemic infectious agents and noninfectious insults. Complete sample selection with complete communication of your diagnostic question(s). Longitudinal and cross-sectional studies using appropriate diagnostic tools for the range of agents of interest.
6. Disease-driven diagnostic testing should be driven by sufficient samples to fully investigate the differential diagnosis.
7. Response to vaccination or vaccination changes in a field trial setting

***Mycoplasma hyopneumoniae*-associated enzootic pneumonia**

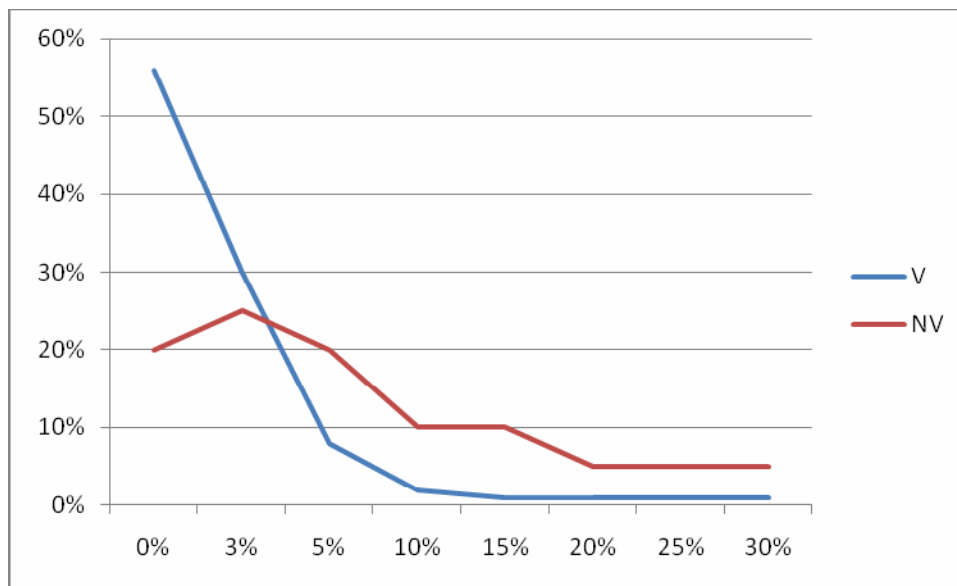
Two scenarios prompt diagnostic testing for Mh. The first is the “not infected” status where the population is allegedly negative for Mh infection; testing is utilized to monitor and affirm the alleged negative status. This scenario is not included in this discussion as it is primarily an epidemiologic process and a mathematics problem.

The second scenario is when herd status is “infected” and where one wishes to determine the impact of infection; that is, to quantify infection or disease impact irrespective of vaccination status. The outcomes to this scenario are either an acceptable level of impact because effects are adequately controlled, or an unacceptable level of impact because there is inadequate control, confounders are present, or errors in assessments or vaccination implementation occur.

Vaccination and therapeutics do not prevent Mh infection, hence at least some pigs will be positive for infection. Nor will all lesions always be eliminated with any intervention. In large populations of animals there is a good chance of finding individual animals with

infection and with lesions. Vaccination or a therapeutic is expected to decrease the number of animals affected as well as the magnitude and duration of lesions. Said another way, in most vaccination trials, one expects to find lesions and infection in vaccinates, but fewer of them. (Example figure 1). Doing complete diagnostic workups on a few poor-doing pigs virtually assures detection of a multitude of endemic pathogens but this does not define the biological or economic impact of these agents on the population.

Figure 1: % of pigs with given % lung involvement in vaccinated (V) and nonvaccinated (NV)



Estimating economic impact of Mh from individual pig diagnostics or single diagnostic tests is futile. Besides the inherent bias of sampling a sick pig, lesions (gross and microscopic) are not pathognomonic. Gross lesions of “cranioventral bronchopneumonia” occur with a variety of agents but are often still considered by many to be grossly compatible with Mh involvement. Histopathology examines a thumbnail sized, 4um slice of tissue to detect presence or absence of a compatible microscopic lesion. PCR is a sensitive and specific test to confirm presence of Mh but it does not differentiate infection from disease. When positive, IHC or FAT confirm Mh in the presence of disease but have drawbacks in sensitivity due to factors inherent in the tests, specimen selection, specimen preservation, and stage of infection or disease. The magnitude of Mh-associated disease in an individual animal cannot be estimated from histopathology, PCR, serology, IHC, or FAT but may be estimated by the extent of gross lesions (after confirmation Mh is involved). So, taken together (a bronchopneumonia with compatible gross and microscopic lesions, positive test for presence of Mh) confirms a role for Mh in the pneumonia. Of course a very basic common question that follows is “what else is there?” Again, thorough evaluation of untreated animals early in the course of symptoms may reveal agents of even greater contributory significance.

Some diagnostic criteria for disease associated with Mh

1. Organism is present: this is an absolute criterion
 - a. PCR is sensitive and specific. A positive test confirms presence of Mh infection, but not causation of disease. The “order of sensitivity” by specimen type (not really a concern in an infected herd) is BAL, airway swab, lung, OF/nasal swab
 - b. Serology (e.g. ELISA) to detect antibody can be useful with caveats:
 - i. Time to seroconversion following infection is inconsistent
 1. Onset of detectable antibody varies from weeks to never
 2. Usually a week or so after symptoms/lesions are present
 3. Higher values and achieved faster if vaccinated
 - ii. Seropositive does not confirm significant impact (disease) from infection
 - c. IFAT and IHC demonstrate organism in an affected airway
 - i. Not a sensitive test
 - ii. The time point sampled in the timeline of infection, active lesions, and resolution
 - iii. Specimen quality/location: fresh, symptomatic, larger affected airways
2. Clinical signs: not specific, preferably includes a knowledgeable veterinarian
 - a. **Objectively** define the clinical syndrome(s) present to avoid bias
 - b. There is wide variation of sensitivity and specificity between observers; some are “better” than others, sometimes, but none are free of bias
 - c. Initially, a cough without much fever with increasing or crescendo over weeks and increasing prevalence of “stall-out/thumping”
3. Gross lesion assessments: lesions are not pathognomonic!
 - a. **Subacute/chronic AV bronchopneumonia is not etiologically specific**
 - b. Pasteurella most frequent co-infection
 - c. Bronchopneumonia has many causes
 - d. Best estimate of severity by quantifying extent of compatible lesions
4. Microscopic lesion assessments often state something like “compatible with but not specific for” for a reason; lesions are not pathognomonic!
 - a. **“Classic” lesion is dependent on timing and absence of confounders**
 - i. Peribronchiolar cuffing with hyperplasia (reactive follicles) of BALT
 - ii. Mucus, neutrophils in airways and adjacent alveoli
 - iii. Examine multiple sections with airways from affected lung
 - iv. Is not “quantitative” as to lesion extent or severity
 - b. **Peribronchiolar cuffing is not etiology-specific**
 - i. Prior or current viral insult (e.g. SIV, PCV2)
 - ii. Chronic bronchopneumonia of a variety of causes
5. Absence of other infectious confounders capable of causing primary pneumonic lesions (e.g. SIV, PCV2, ascarid migration, bacteria). This again requires a complete herd evaluation using all available resources as well as carefully considered plan for laboratory testing.

It should be clear that the magnitude of impact of Mh in a herd cannot be estimated from laboratory specimens. If the gross lesions are extensive and consistently present, a greater impact is suspected in clinically affected pigs. This still does not assess impact on a herd basis. There are various diagnostic tools available to swine practitioners for Mh specifically and disease generally (as previously discussed). Balancing the value of these tools with ability to make better decisions on the farm is a challenge for most of us. It is indeed a mind's challenge to comprehend the dozens of factors changing dynamically over time within each individual in a population of many. To be effective in the workplace, veterinarians use practicality to package information and execute processes in a way as to "keep it simple". Biology, on the other hand, is not simple.