

OHIO STATE UNIVERSITY EXTENSION



OHIO VETERINARY NEWSLETTER

April 22, 2016

Veterinary Extension

Vol 42, No 5

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Testing Protocols for Disease Surveillance in Poultry

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Any surveillance program is intended to be an early warning system that detects the infection as early as possible to allow for timely control and eradication of the infectious agent. The poultry industry relies heavily on surveillance to keep certain diseases out of the population. Avian Influenza (AI), Avian Mycoplasma, and Salmonella are examples of such diseases. Surveillance is an intricate and complicated process that can be different in different diseases and in different situations. In this article we will try to review the general principals of surveillance programs in poultry and try to extract the basic concepts by which we can critically examine any surveillance program.

TEST CHARACTERISTICS

The characteristics of any laboratory test dictate how it can be used in a surveillance program. Before we review the surveillance programs, let's first discuss some basic concepts about laboratory tests and their characteristics. A disease status for an individual in a population can be either disease positive (D+) or disease negative (D-). Similarly, when a test is used on an individual in the population, the test results can be a positive result (T+) or negative result (T-). However, and due to inherent flaws in the testing assays, the result is almost never 100% accurate. A disease positive individual (D+) may give a negative test result (T-) and in this case a test result is called a False Negative (FN). Also, a disease negative individual (D-) may give a positive test result (T+) and in this case a test result is called a False Positive (FP).

The FN and the FP rates for a certain test are influenced by test parameters; sensitivity and specificity. Sensitivity quantifies the ability of the test to avoid false negatives. So, when the sensitivity of a test is high, it means that there is a high chance that the sample is actually negative when the test result is negative. Specificity on the other hand quantifies the ability of the test to avoid false positives. So, when the specificity of a test is high, it means that there is a high chance that the sample is actually positive when the test result is positive. Sensitivity and specificity are typically inversely correlated for a given test. In other words, tests with high sensitivity typically have low specificity and vice versa (there are exceptions to this rule). Tests with high sensitivity and low specificity (sensitive tests) are prone to FP, and tests with high specificity and low sensitivity (specific tests) are prone to FN.

SURVEILLANCE SETUP

There is more than one way to utilize both sensitive and specific tests in a surveillance program; a common way to do that is to use a "series testing" setup. In this setup, a

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sensitive test is used as a screening test first, and then any positive samples on the screening test are tested for a second time with a specific confirmatory test. It is imperative that the tests in the series testing surveillance program are used in that order, the sensitive screening test first followed by a confirmatory specific test for the positive samples. This series testing setup achieves a high degree of certainty in a couple of situations; first, a negative sample on the screening test is considered negative with high probability. Second, a positive sample on both the screening and the confirmatory test is considered positive with high probability. However, there is one situation in which the results of a series testing are considered suspected positive. This situation is when the sample is positive on the screening test and negative on the confirmatory test. This assumes the sample is weakly positive, just enough for the sensitive test to pick it up, but not enough for the specific test to confirm it. In this situation a second confirmatory test is required to clear up the uncertainty.

SELECTING SURVEILLANCE TESTS

We will use two poultry diseases as examples of how to select among multiple available tests to build a good surveillance program. Avian Mycoplasma is one of the costliest diseases facing the poultry industry. Surveillance programs are in place to detect the infection particularly in breeding flocks. As previously stated, the goal of any surveillance program is to act as an early warning system that detects the infection as early as possible to allow for timely control and eradication of the infectious agent. For Avian Mycoplasma, a typical surveillance program utilizes a combination of the following serological tests to achieve that goal: 1.) the Serum Plate Agglutination TEST (PA), 2.) Enzyme-Linked Immunosorbent Assay (ELISA) and 3.) Hemagglutination Inhibition test (HI). Avian Influenza is another disease that is really challenging the poultry industry. AI is transmitted horizontally, hence all kinds of birds need to be tested before they are moved. For Avian Influenza surveillance programs, two serological tests are commonly used: 1.) the Enzyme-Linked Immunosorbent Assay (ELISA) and 2.) the Agar Gel Immunodiffusion test (AGID). In addition to a serological test, polymerase chain reaction (PCR) based testing is a good option to consider in a surveillance program.

It is widely accepted that among serological tests for mycoplasma, PA is the most sensitive, and HI is the most specific, while ELISA is in the middle for both sensitivity and specificity. So an ideal surveillance program for mycoplasma would be to use PA (sensitive) as a screening test and to use HI (specific) as a confirmatory test. Since mycoplasma is a vertically transmitted disease, this testing protocol should be administered on all breeders at least once at placement, once before the onset of production, and repeated every 3 – 4 weeks during the egg laying period.

Regarding the two serological tests used for AI, ELISA has the higher sensitivity and the AGID is considered the more specific test. So an ideal surveillance program for AI would be to use ELISA (sensitive) as a screening test and to use AGID (specific) as a confirmatory test. Since AI is a horizontally transmitted disease, this testing protocol should be performed for all birds at least once 2–3 weeks before each time they are moved.

PCR is both sensitive and specific, and could be used as a screening and as a confirmatory test. However, PCR, unlike serology, is unable to detect past infections in the flock. So, unless the infection is current and the agent is still actively replicating in the flock, PCR cannot detect the infection. Also, PCR is more expensive and requires higher technical capabilities than serology. For these reasons, serological tests are still the preferred option for screening, but PCR can be used as a confirmatory test.

SURVEILLANCE COMMON MISTAKES AND PITFALLS

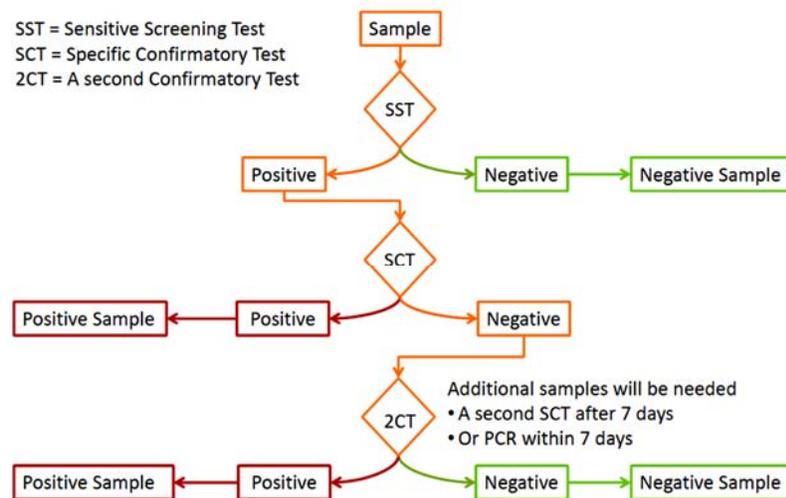
The most common mistake is to skip the use of a screening test and rely solely on a specific test. Using a less sensitive test as the sole surveillance test makes the surveillance program prone to false negatives. In other words, it makes it possible for an actually positive sample to be missed and passed as a negative sample. This may allow the infectious agent to continue to circulate in the commercial poultry population unnoticed. So, using a less sensitive test as the screening test defies the purpose of the surveillance program. To avoid this pitfall, a sensitive screening test should always be the first step of any surveillance program.

Another common gap in surveillance programs as mentioned before is in the situation where the sample is positive on the screening test and negative on the confirmatory test. It is very common in this situation to consider the sample as a negative sample while the proper classification should be “suspect sample”. In this situation, a second confirmatory test is always recommended.

SURVEILLANCE PROTOCOL

A good, simple and efficient surveillance protocol can be as follows:

- Original Sample: Tested by Sensitive Screening Test → Negative → Test is negative and the sample is considered negative.
- Original Sample: Tested by Sensitive Screening Test → Positive → Confirmatory Specific Test → Positive → Test is positive and the sample is considered positive.
- Original Sample: Tested by Sensitive Screening Test → Positive → Confirmatory Specific Test → Negative → A second confirmatory test is required: Swabs for PCR (and/or isolation) within 7 days from the first sample or a second serological Confirmatory Specific Test sample after 7 days from the first sample.
- Second Sample: Second confirmatory test → Negative → Test is negative and the flock is considered. Negative
- Second Sample: Second confirmatory test → Positive → Test is positive and the flock is considered positive.



In this review we used two examples for surveillance programs in poultry, one for a vertically transmitted disease, mycoplasma, and the other for a horizontally transmitted disease, AI. The purpose of using these examples is to review the basic concepts of surveillance programs and give the reader the tools to critically examine any surveillance program for any disease. And briefly, these concepts include the use of a sensitive test for screening first, and then follow that with a specific confirmatory test in case the screening result was positive. Also, don't overlook the situation where a screening test is positive and the confirmatory test is negative, in that situation a second confirmatory test is needed to clear the uncertainty.

Q&A Session

QUESTION: It is true that pasteurization can affect milk nutritional composition, cause allergic reactions, and lactose intolerance?

ANSWER: Many studies show no significant difference when comparing nutrient content from pasteurized and unpasteurized milk. The pasteurization process will kill pathogens responsible for causing diseases such as Salmonellosis, Listeriosis, Campylobacteriosis, Tuberculosis, and Brucellosis to cite some. It is important to note that nonpathogenic bacteria may still be present at very low numbers and capable of causing pasteurized milk to spoil. This is why it is crucial to keep milk refrigerated, and why even pasteurized milk eventually goes sour in the refrigerator. All of the nutritional benefits of milk can be obtained by drinking pasteurized milk without the risk of disease associated with drinking raw milk.

Pasteurization doesn't cause allergic reactions - the milk proteins which cause allergic reactions in people sensitive to dairy products are present in both products – raw and pasteurized milk. Same is true for lactose intolerance (not to be confused with milk allergy). Intolerance to lactose is caused by insufficient production in the body of an enzyme necessary to break down the lactose – that is present at the same concentration in both raw and pasteurized milk.

Research

Vieira-Neto, A., Lima, F. S., Santos, J. E., Mingoti, R. D., Vasconcellos, G. S., Risco, C. A., & Galvao, K. N. (2016). **Vulvovaginal laceration as a risk factor for uterine disease in postpartum dairy cows.** *Journal of Dairy Science*. Advanced online publication. doi: 10.3168/jds.2016-10872

BACKGROUND: Several risk factors are recognized for both metritis and purulent vaginal discharge (PVD), but information is lacking about the association between vulvovaginal laceration and uterine disease. The authors hypothesized that cows having vulvovaginal laceration at calving would be associated with greater incidence of uterine diseases and impairment of reproductive performance.

PURPOSE: The objective was to evaluate the association between vulvovaginal laceration and the incidence of metritis and purulent vaginal discharge. The secondary objectives were to evaluate the association between vulvovaginal laceration and cyclicity, and reproductive performance.

RESULTS: Cows with vulvovaginal laceration score (VLS) 2 had greater incidence of metritis than cows with VLS 0 (69.1 vs. 42.4%), and cows with VLS 1 tended to have greater incidence of metritis than cows with VLS 0 (52.0 vs. 42.4%). Cows with VLS 2 had greater incidence of PVD than cows with VLS 0 (56.5 vs. 43.1%). A lower proportion of cows with VLS 2 than VLS 0 were cyclic by 64 DIM (70.0 vs. 86.8%). A lower proportion of cows with VLS 2 than VLS 0 were pregnant at 60 d after first AI (28.7 vs. 33.6%). Proportion of pregnant cows at 60 d after AI tended to be lower for VLS 1 than VLS 0 (28.4 vs. 33.6%). Hazard of pregnancy by 300 DIM was not affected by VLS. Hazard of pregnancy was decreased for cows with metritis, PVD, and anovular cows.

CONCLUSIONS: The authors concluded that vulvovaginal laceration was associated with uterine disease and cyclicity, which were negatively associated with reproductive performance. Vulvovaginal laceration was recognized as a risk factor for uterine disease postpartum.

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Cummins, C., Lorenz, I., & Kennedy, E. (2016). **Short communication: The effect of storage conditions over time on bovine colostrum immunoglobulin G concentration, bacteria, and pH.** *Journal of Dairy Science*. Advanced online publication. doi: 10.3168/jds.2015-10276

BACKGROUND: In Ireland, >90% of dairy farmers store colostrum, up to 17% of whom store colostrum at room temperature. Storing colostrum at >4°C allows growth of bacteria and a reduction in pH. The effect of colostrum storage at ≥4°C on IgG concentration,

bacteria, and pH has not previously been investigated on a single sample set; hence, it is difficult to ascertain the key changes and interactions that may occur.

PURPOSE: The objective was to measure the effect of various storage conditions over time on IgG concentration, bacteria, and pH in colostrum of Holstein-Friesian dairy cows in Ireland.

RESULTS: Storage conditions did not affect the IgG concentration of colostrum. Bacterial growth was most rapid in the first 6 h of storage, reducing thereafter, but bacteria multiplied at a significantly greater rate when stored in warmer conditions (i.e., >4°C). The pH of colostrum was not significantly altered when stored at temperatures <13°C, but when stored at 20°C the pH significantly decreased after 24 h of storage. Storing colostrum in warmer conditions significantly alters both total bacteria count and pH; consequently, colostrum should be stored at ≤4°C.

CONCLUSIONS: Colostrum is significantly affected by storage conditions and duration. Bacteria and pH are significantly altered and changes are more rapid when colostrum is stored >4°C. The first 6 h postcollection are critical, as a gross increase in TBC occurs. The rate of bacterial growth is greater with higher storage temperatures, and thus colostrum should be refrigerated immediately after collection to minimize bacterial growth. Although IgG is not affected by colostrum storage or duration, the rate of absorption in the calf may be affected by the bacterial and pH, and thus further investigations need to be conducted.

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Godden, S. M., Royster, E., Knauer, W., Sorg, J., Lopez-Benavides, M., Schukken, Y., ... French, E. A. (2016). **Randomized noninferiority study evaluating the efficacy of a postmilking teat disinfectant for the prevention of naturally occurring intramammary infections.** *Journal of Dairy Science*. Advanced online publication. doi: 10.3168/jds.2015-10379

BACKGROUND: Formulating teat disinfectants with chemicals that naturally occur in milk is an interesting opportunity for the udder health solutions industry, because concerns about residues in milk are minimized. Glycolic acid was recently approved by biocidal products regulation for use as a germicide in teat dips. However, glycolic acid alone shows limited germicidal efficacy, hindering its chances to meet a teat disinfectant's minimal requirements for the product's intended purpose. Field-based studies are needed to measure the efficacy of postmilking teat disinfectants using germicides that naturally occur in milk, as well as to evaluate safety to ensure that the product is not irritating to teat skin.

PURPOSE: The aim was to measure the efficacy of a novel glycolic acid-based teat disinfectant, OceanBlu Pre Post, when applied postmilking. The primary objective was to demonstrate noninferiority of this experimental (EX) test product when compared with a previously proven iodine-based positive control (PC) product with regard to the incidence of new intramammary infections (NIMI) that occurred under natural challenge conditions on a commercial dairy farm. Secondary objectives included describing the effect of treatment on prevalence of intramammary infections (IMI), somatic cell count (SCC), and teat condition throughout the trial period.

RESULTS: The results indicate that the glycolic acid-based EX product (OceanBlu Pre Post, DeLaval) was noninferior to the previously proven iodine-based PC product (Bovadine I-Tech II, WestAgro) for the prevention of naturally occurring intramammary infections. Also, no overall difference was found between the 2 products on the incidence of NIMI, the risk for presence (prevalence) of IMI, SCC, or measures of teat skin condition.

CONCLUSIONS: The authors concluded that OceanBlu Pre Post can be considered an effective postmilking teat disinfectant, as well as safe, in that the product was not irritating to teat skin and, overall, did not negatively affect skin condition, as compared with the

positive control group. Additional studies are needed to ensure that results are repeatable under different management and seasonal conditions.

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April 27-28, 2016
OVMA, Powell, Ohio
No cost, but registration is required.

Dairy Cattle Welfare Symposium

Intersection of Best Practices and Sustainability
May 20-21, 2016
Ohio Union, Columbus, Ohio
(limited to 265 attendees; 30 spots available to students)

Ohio Dairy Health and Management Certificate Program

Spots are always available for specific module plan.

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