Testing Protocols for Diseases Surveillance in Poultry

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 review the general principals of surveillance programs in poultry and 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 may give a negative test result (D+/T-), and in this case, a test result is called a False Negative (FN). Also, a disease negative individual may give a positive test result (D-/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 often 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 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 SURVEILANCE TESTS

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. 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 because this disease is transmitted vertically. 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 either 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, particularly as a secondary confirmatory test as explained below.

SURVEILANCE COMMON MISTAKES AND PITFALLS

The most common mistake is to skip the use of a sensitive 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 must 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 would be as follows:

- Original Sample: Tested by Sensitive Screening Test → Negative → The sample is considered negative.
- Original Sample: Tested by Sensitive Screening Test → Positive → Specific Confirmatory Test → Positive → The sample is considered positive.
- Original Sample: Tested by Sensitive Screening Test → Positive → Specific Confirmatory Test → Negative → The sample is considered suspect and a Second Confirmatory Test is required: Swabs for PCR (and/or isolation) within 7 days from the original sample or a second serological sample after 7 days from the original 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.
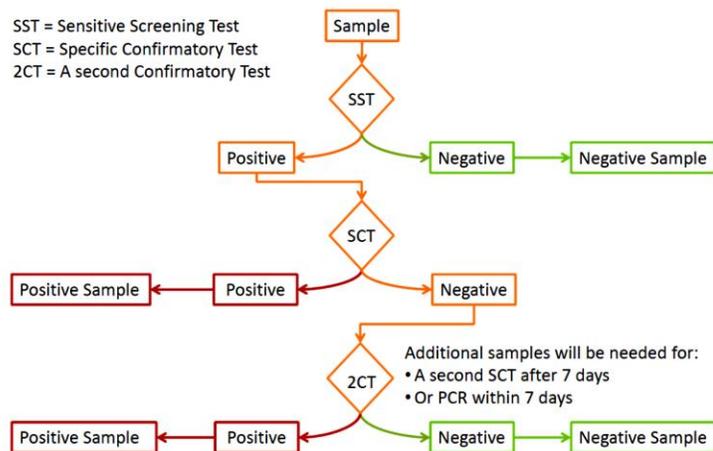
In this review we used two examples for surveillance programs in poultry, one for a vertically transmitted disease, Avian Mycoplasma, and the other for a horizontally transmitted disease, AI. The purpose of using these examples is to review the basic concepts of surveillance programs and give the

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 when the screening result is positive. Also, don't overlook the situation where a screening test is positive and the confirmatory test is negative; in that situation the sample should be considered suspect and a second confirmatory test is needed to clear the uncertainty.