New York State Cattle Health Assurance Program
Johne's disease in Cattle - Article 6

This is the sixth article in a series presenting current information regarding Johne's disease in cattle. It is directed toward helping veterinarians and their clients prevent or control this disease and was adapted with permission from the original 1999-2000 series presented by the AABP Food Safety Committee. Content was edited and reviewed by the National Johne's Working Group and endorsed by the USAHA.

Johne's Disease Diagnostic Tests – Fecal Culture

Part 3 of 4 on the topic of Johne's disease testing

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Culture methods

Mycobacterium avium subs. *paratuberculosis* (*Map*) is the microbe that causes Johne's disease. For decades, culture of fecal samples for *Map* has been the anchor of Johne's disease diagnosis. Two major methods are currently used:

1. Conventional culture on solid media slants, which detects growth by colony formation.
2. Radiometric culture in liquid media, which detects growth by release of labeled carbon 14.

Despite extensive reliance on this test, conventional culture methods are not standardized and laboratories vary in their capabilities to provide the most accurate and highest quality results. Two major improvements have increased the sensitivity of conventional culture: centrifugation to concentrate the bacteria and double incubation with antibiotics to decrease contamination by other microbes. NVSL **(what is this?)** recommends that laboratories use a referenced centrifugation method versus sedimentation.

Conventional culture

Map is difficult to grow and the conventional process requires several steps over a period of several days. Centrifugation concentrates *Map* and greatly improves detection, but also increases the potential to contaminate organisms. Samples are decontaminated to selectively minimize growth of competing organisms. Incubation with antibiotics further reduces the number of other contaminant organisms, but also inhibits *Map* to some extent.

Typically, four tubes of Harold's egg yolk agar are inoculated and incubated for 12 to 16 weeks. Samples are routinely checked for *Map* growth throughout the period. *Map* is identified based on colony morphology, acid fast staining, slow growth rate, and dependence on mycobactin J growth factor.

Some laboratories perform additional DNA testing to confirm positive cultures with an IS900 sequence probe that is specific for most *Map* isolates.

Radiometric culture

This method is semi-automated and requires sophisticated instrumentation and radioisotopes. Its sensitivity is similar to culture, but it has a shorter

growth time of only four to seven weeks. Growth must be confirmed by IS900 probe and is used mostly in research facilities.

Culture Results

Normally mycobacteria are clumped and form a colony unit when they grow. *Map* growth on solid media is measured by counting the number of visible colony forming units or CFUs.

To better quantify results, NVSL recommends that labs report culture results as CFUs per gram of feces, not simply as positive or negative growth. If a standard centrifugation method is used, CFU counts range from 1 to 100 per tube, with 100 equivalent to TNTC (too numerous to count). Total CFUs for a standard four-tube set can range from 1 to 300 (one tube is only for ID confirmation), or TNTC, per gram of feces processed. The lower limit of detection for centrifugation fecal culture is about 10 CFU per gram of feces.

> To reduce sources of variation, practitioners should confirm that their lab uses a centrifugation method, reports CFUs / gram of feces, confirms questionable *Map* isolates by IS900 and is accredited in the NVSL check test. *Accredited labs are listed at http://www.usaha.org*

Accuracy of fecal culture

Recall the four stages of progression for Johne's disease; animals in Stage I, and many in Stage II, do not shed enough *Map* to be detected and a false negative test results. However, fecal culture tends to be more sensitive than serological tests. Low levels of organisms can be detected in some Stage II animals, which are typically negative on ELISA. Based on decades of use, the sensitivity of culture is estimated at 30 to 50%. When employed in a typical herd, or for the National Johne's Herd Status Program, the National Johne's Working Group expert panel recommends a sensitivity estimate of 40% for fecal culture.

The specificity of conventional fecal culture can be 100%. With a specificity of 100%, a hallmark advantage to fecal culture is that a positive result indicates true infection. A 100% specificity assumes that sample collection and procedures were performed properly. Cross contamination of fecal samples can occur and strict precautions should be followed during collection, handling, and processing.

A 100% specificity also implies that "once detected, means infected" and that an episode of *Map* pass-through has not occurred. Although Whitlock cultured *Map* from feces of cattle less than 12 hours after feeding them manure from a high CFU (TNTC) shedder, the extent that this pass-through phenomenon occurs naturally on farms has not been determined. Pass-through may be possible in heavily contaminated environments, but such high-risk environments also imply that many animals are already infected and are shedding high numbers of microbes.

A more complete history and risk assessment is required to determine the actual plausibility and occurrence of pass-through events in a specific herd, which can affect the interpretation of culture results for that herd.

The influence of prevalence on interpreting fecal culture results

With a specificity of 100%, the chance that a positive culture is correct is almost always 100%, regardless of disease prevalence. The predictive value for a positive test is also always 100% when properly performed.

The influence of prevalence on the confidence in an individual negative fecal culture result is similar to the ELISA. In a low prevalence herd or situation (±1%), the chance that a negative culture indicates non-infection in a mature animal is 99.7%. In a high prevalence situation, i.e. 30%, the chance that a negative culture indicates non-infection in a mature animal drops to 79%.

> When herd prevalence or likelihood of infection is unknown, the level of confidence for a negative culture in a mature animal can not be judged. A negative culture also means little in an immature individual. In these situations, a negative culture should be interpreted as "infection not detected," not *as* "infection not present".

Use of fecal culture

Of all tests that are currently available, culture provides the most accurate, definitive, and quantitative information about the status of an individual or herd. For this reason it has the highest value to:

1. Definitively confirm infection in the herd.
2. Pursue more definitive status of infection in ELISA positive cattle.
3. Aggressively detect infected cattle, especially if used strategically with serology.
4. Quantify shedding status of individual cattle or groups.

Because specificity and sensitivity of culture is higher than serological tests, it is the best choice for confirming positive serological test results.

Occasionally, an infected animal with a positive ELISA result has a negative result on a single fecal culture test. With time, repeat cultures should detect shedding of *Map.*

Fecal culture detects infection in immature animals that have unusually advanced infection and shed *Map* at 12 to 24 months of age. However, routine use in immature animals is not recommended unless specific circumstances dictate the need for maximum early detection.

Three disadvantages likely preclude high volume use of culture in Johne's control strategies. High volume culturing requires laboratories to make substantial investments in equipment, space, and technical time that are well beyond existing capacities or resources. Culture requires 12 to 16 weeks to complete and therefore bars its use when information is needed quickly. Conventional culture can be expensive when sampling large numbers of animals -- across the U.S. costs range from $7 to $25 per sample.

An advantage of culture is that fecal shedding can be detected earlier, generally in Stage II, and usually at a low level (10 CFU/gram). Colony counts increase in animals that progress to Stage III and IV and that eventually test

as TNTC.

Assessing disease stage is another advantage of fecal culture. TNTC animals have disseminated infection that progresses toward clinical disease when high levels of mycobacteria (10^3 to 10^8 organisms per gram of feces) are shed.

High CFU counts also correlate with a high risk that *Map* will be secreted in milk and colostrum or infect the fetus. TNTC results are often reported by eight weeks of culture, yet too often, these cattle have already been removed for clinical disease. Cattle that progress more slowly can have TNTC results on repeated fecal samples.

In contrast, low shedders (i.e. 1 to 10 CFU per gram) appear to have intermittent shedding patterns and may not be positive when cultured a second or third time. Long-term follow-up studies by Whitlock suggest that 30% of low shedders progress to Stage III or IV in two to four years.

Thus, culture results with standardized colony counts are more useful to differentiate animals in advanced infection than to differentiate those in early infection. Culture results can help prioritize animal removal or management decisions when eradication is not the immediate goal. Low-level fecal shedders may be candidates for management or monitoring, whereas high shedders are candidates for immediate removal or separation.

Summary of fecal culture diagnosis

1. Be certain that the diagnostic laboratory uses a centrifugation method, reports accurate CFU results, and has passed the NVSL check test for Johne's fecal culture.
2. Fecal culture is considered the definitive test for *Map* infection.
3. Because of its high specificity, virtually 100% of correctly processed positive culture results can be interpreted as true infection.
4. Fecal culture can detect infection earlier than ELISA and has an estimated sensitivity of 40% in infected herds.
5. Fecal culture is recommended for confirmation of the accuracy of a positive serological test result.
6. CFU counts correlate with advancing disease stages, risk for shedding in colostrum or milk, and risk for fetal infection.
7. In immature cattle, or in unknown prevalence environments, negative results should be interpreted as "infection not detected" not *as* "infection not present".
8. Fecal culture is recommended in immature cattle only when aggressive detection is essential.
9. Fecal culture may be used to control Johne's disease in vaccinated herds and to monitor progress.