

## **Johne's disease, Article No. Four**

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*This is the third part of the fourth article in a series presenting the most current information and methods about Johne's disease in cattle. **The series is directed toward veterinarians helping clients prevent or control this disease.** Information was edited and reviewed by the National Johne's Working Group.*

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### **Johne's Disease Diagnostic Tests – Fecal Culture Part 3 of 4**

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#### **Culture methods**

*Mycobacterium avium* subs. *paratuberculosis* is the name of the microbe that causes Johne's disease. In this article it will be referred to as MAP. Culturing fecal samples for MAP has been the anchor of Johne's disease diagnosis for decades. There are two major methods currently in use:

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

Despite our 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 a double incubation with antibiotics to decrease contamination by other microbes. NVSL recommends that laboratories use a referenced centrifugation method versus sedimentation.

#### **Conventional culture**

MAP is fastidious and difficult to grow. Several steps are involved in the

conventional process. Centrifugation concentrates MAP and greatly improves detection, but increases contaminating organisms as well. Samples are decontaminated to selectively minimize growth of competing organisms. Incubation with antibiotics further reduces contaminating organisms, but 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 do 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. It has similar sensitivity to culture but has the advantage of a shorter growth time of only 4-7 weeks. Growth must be confirmed by IS900 probe. It is used mostly in research facilities.

## Culture Results

Normally these 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 positive or negative growth. If a standard centrifugation method is used, CFU counts range from 1-100 per tube, with 100 equivalent to TNTC (too numerous to count). Total CFUs for a standard four-tube set could 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.

With multiple sources of variation practitioners should be sure their lab is using a centrifugation method, reports CFUs / gram of feces, confirms questionable MAP isolates by IS900, and participates in the NVSL check test.

## Accuracy of fecal culture

Recall the four stages of progression for Johne's disease. Animals in Stage I and many in Stage II will not shed enough MAP to be detected and will have a false negative test result. However fecal culture tends to be more sensitive than serological tests. Low levels of organisms can be detected in some Stage II animals, the majority of which are negative on ELISA. Based on decades of use, the sensitivity of culture is estimated to be 30-50%. When employed in a typical herd, or for the

National Johne's Herd Status Program, the National Johne's Working Group expert panel recommended 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 a true infection. A 100% specificity assumes that sample collection and procedures have been performed properly. Cross contamination of fecal samples can occur and strict precautions should be followed during collection, handling, and processing.

This 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 <12 hours after feeding them manure from a high CFU (TNTC) shedder, the extent to which this pass through phenomenon occurs naturally on farms has not been determined. It 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 would be required to determine the actual plausibility and occurrence of pass through events in a specific herd. Pass through could effect the interpretation of culture results for that herd.

## The influence of prevalence on interpreting fecal culture results

With a specificity of 100% there is little doubt about interpreting a positive result. The chance that a positive culture is correct is nearly always 100%

regardless of disease prevalence. The predictive value for a positive test is always 100% when properly performed.

The influence of prevalence on the confidence practitioners can have in an individual negative fecal culture result is similar to the ELISA. In a low prevalence herd or situation ( $\pm 1\%$ ), 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 the herd prevalence or likelihood of infection is unknown, it is not possible to judge the level of confidence for a negative culture in a mature animal. 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 currently available tests, 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 for some uses:

1. Definitive confirmation of infection the herd
2. Pursue more definitive status of infection in ELISA positive cattle
3. Aggressive detection of infected cattle, especially if used strategically with serology
4. Quantify shedding status of individual cattle or groups

The higher specificity and sensitivity of culture over serological tests make it the best choice for confirming positive serological test results.

Occasionally, a truly infected animal with a positive ELISA result will have a negative result on a single fecal culture test. With time, repeat cultures should detect shedding of MAP.

Fecal culture will detect infection in immature animals that have unusually advanced infection and be shedding MAP at 12-24 months of age. However its routine use in immature animals is not recommended unless specific circumstances dictate need for maximum early detection.

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

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

Assessing disease Stage is another advantage of fecal culture. Animals with TNTC results have disseminated infection, are progressing toward clinical

disease, and are shedding high levels of mycobacteria ( $10^3$  to  $10^8$  organisms per gram of feces)!

High CFU counts also correlate with high risk that MAP will be secreted in milk and colostrum *or* infect the fetus. TNTC results are often reported by 8 weeks of culture, *yet too often*, these cattle have often 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. 1-10 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 2-4 years.

Thus, culture results with standardized colony counts can be useful to differentiate animals more likely in advanced compared to early infection. This 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.

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### Summary of fecal culture diagnosis

1. Be certain that the diagnostic laboratory is using a centrifugation method, reports accurate CFU results, and has passed the NVSL check test for Johne's fecal culture.
2. Fecal culture is considered to be the definitive test for MAP infection.
3. Because of its high specificity, virtually 100% of positive culture results, when processed correctly, 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 monitor progress.