

Navigating Johne's Disease Testing

-- Beef Cattle and Small Ruminants --

This article is intended for veterinarians and focuses on applying diagnostic tests for Johne's disease to assist in guiding testing strategies for their producer clients. While producers may also benefit from this information, those seeking more detailed background on Johne's disease are encouraged to consult additional online resources provided at the end of this article (see References).

Introduction to Johne's Disease

Johne's disease, caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP) – also referred to as paratuberculosis – is a chronic, progressive, and ultimately fatal gastrointestinal disease that primarily affecting domestic ruminants (cattle, sheep, goats, camelids and buffaloes) and wild ruminants (such as deer, elk, and bighorn sheep). Currently there is no effective treatment or cure, and no commercially available vaccine in the United States. Although historically associated with dairy cattle, Johne's disease is becoming an increasing concern in beef herds [USDA, 1997; Johnson et al. 2022].

The Pathogen and Disease Progression:

- **Causative Agent:** *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a hardy bacterium that can survive in the environment for extended periods (up to a year), though it only replicates within the host's macrophages.
- **Infection in Young Animals:** Calves, lambs, and kids are most susceptible, typically acquiring the infection through ingestion of manure-contaminated feed, water, milk, or directly from infected dams. In utero transmission is also possible.
- **Subclinical Phase:** Infected animals remain clinically normal for months to years while MAP colonizes intestinal tissues.
- **Clinical Signs:** Clinical signs typically manifest in adult animals (usually >18 months) and is characterized by progressive weight loss despite a good appetite. In cattle, chronic diarrhea is common in later stages of disease. Submandibular edema ("bottle jaw") due to protein loss may also occur in advanced stages. In small ruminants, sheep tend to show clinical signs less frequently than goats, and diarrhea is less common in both. For instance, only 20% of infected sheep show diarrhea at the end stage, and goats often do not exhibit diarrhea at all.

- **Transmission:** Fecal-oral transmission from infected adults to youngstock is the primary route. Shedding of MAP in feces increases over time, and MAP may also be present in milk and colostrum. Introduction of silently infected animals into a herd is a major risk factor.
- **Environmental Contamination:** Manure (feces) is the main vehicle for environmental spread. MAP can persist in soil and water for extended periods, contributing to herd-level transmission risks. [Grant, 2010]

Rising Concern in Beef Cattle:

Surveys and diagnostic data suggest an increasing prevalence of MAP infection in U.S. beef herds, particularly in purebred operations with frequent animal movement. The current prevalence in U.S. sheep and goat herds remains unknown (NAHMS, 2023).

Defining Your Testing Goals:

Clarifying the objective of testing is essential before selecting a diagnostic approach. Consider whether the goal is to:

- Initially screen for MAP presence in the herd
- Estimate herd-level prevalence
- Control spread within a known infected herd
- Work toward eradication from the herd
- Diagnose individual cases
- Perform pre-purchase risk reduction

Your testing goal will guide the most appropriate type and scope of diagnostics.

Diagnostic Tools Available

Our laboratory offers a suite of diagnostic options to support various management objectives for Johne's disease. The choice of test depends on the specific context and goals of testing.

1. Organism Detection Tests (Detect MAP directly)

- **Polymerase Chain Reaction (PCR):**
 - **Principle:** Detects MAP DNA (targeting IS900 sequence) in manure or tissue. PCR identifies both live and dead organisms.
 - **Turnaround Time:** 1-7 days
 - **Sensitivity & Specificity:**

- High sensitivity (~95%) for detecting moderate-to-heavy shedders
- Moderate sensitivity (~75%) for low-level shedders or early-stage infections
- Specificity near 100% (very low false-positive rate). (Cornell, accessed 2025-05-16)
- **Applications:**
 - **Herd Screening (Pooled PCR):** Cost-effective in low-prevalence herds. Lab pools 5 manure samples per test; submit individual samples with animal ages to allow efficient pooling in descending age order (Kalis 2000).
 - **Individual Animal Diagnosis:** Useful for clinical suspect animals.
 - **Post-mortem Diagnosis:** PCR can be run on intestinal contents.
 - **Culture Confirmation:** PCR can confirm that MAP was cultured (although WSVL does not offer culture – see below).
- **Sample Submission:** Submit fresh manure (avoid snap-top tubes to prevent contamination) or small intestinal contents.
- **Culture:**

Due to MAP's extremely slow growth – colonies may take 6-12 weeks to appear and up to 6 months for full incubation – **WSVL does not offer culture testing** for MAP.

2. Antibody Detection Tests (Detect immune response)

- **Enzyme-Linked Immunosorbent Assay (ELISA):**
 - **Principle:** Detects MAP-specific antibodies in serum. Antibody levels increase in later stages of infection.
 - **Turnaround Time:** 1-3 business days
 - **Sensitivity & Specificity:**
 - Specificity: High (97-99%), low false-positive rate
 - Sensitivity: Lower in early infection due to delayed antibody response (Nielsen & Toft, 2008)
 - **Applications:**

- **Herd Screening:** Especially cost-effective for large herds.
- **Identifying Potential Shedders:** Higher antibody levels are often associated with active shedding (later stages of infection).
- **Pre-purchase Testing (Buyer Perspective):** While a negative ELISA does not guarantee freedom from infection, it lowers risk. **However, a positive ELISA in an animals from a low-prevalence herd may represent a false positive and should be interpreted with caution.**
- **Sample Submission:** 2-3 mL of blood in a red-top tube (or serum separator tube). **Discounted pricing** is available when more than five samples from the same herd are submitted together (see our [Fee Schedule](#)).

3. Post Mortem (Necropsy)

Necropsy remains an important diagnostic tool in identifying Johne's disease, especially in animals with chronic weight loss or other compatible clinical signs. Post mortem examination allows direct visualization and sampling of characteristic lesions in the ileum and associated lymph nodes.

- **Who to Submit:** Animals >18 months old with a history of progressive weight loss, with or without diarrhea, are the most informative candidates.
- **Gross Lesions:** Thickening of the terminal ileum and enlarged mesenteric lymph nodes may be observed in advanced cases.
- **Diagnostic Testing:** PCR testing can be performed on intestinal contents or affected tissues to confirm MAP presence. Histopathology of formalin-fixed tissues (ileum and ileocecal lymph nodes) is also useful to detect granulomatous inflammation consistent with Johne's disease.
- **General Value:** Beyond Johne's disease, necropsy can help identify other causes of wasting and chronic illness, making it a valuable part of herd health investigation.

Understanding the Impact of Herd Prevalence

The prevalence of Johne's disease in a herd significantly affects the interpretation and reliability of diagnostic results:

- **Low Prevalence Herds:** In herds with a low estimated prevalence, a **positive ELISA** result is more likely to be a **false positive**, while a **negative result** is more likely to be **accurate**. In these herds, more sensitive tests like PCR may be preferred for initial screening.
- **High Prevalence Herds:** In herds with higher prevalence, a **positive ELISA** result is more likely to reflect **true infection**, but **negative results** may miss infected animals that have not yet mounted a detectable antibody levels response.
- **Unknown Prevalence:** When prevalence is unknown, test results should be interpreted cautiously. Follow-up testing with a different method (e.g., PCR confirmation of ELISA-positive animals) is advised.

Determining Herd Status

Current Johne's tests have limitations in sensitivity and specificity, especially in early stages of infection. As a result, **large sample sizes are needed** to determine with confidence whether a herd is free from infection.

Example Testing Thresholds (for 95% Confidence):

- **ELISA**
 - Assumptions: 94% sensitivity, 98% specificity, 6.5% within-herd prevalence
 - **Minimum animals to test:** 126
 - **Expected false positives:** Up to 5
 - **Note:** In herds with ≤ 125 head, all animals must be tested to achieve equivalent confidence.
- **PCR**
 - Assumptions: 95% sensitivity, 99.5% specificity, 6.5% within-herd prevalence
 - **Minimum animals to test:** 68
 - **Expected false positives:** About 1

Pooled Fecal PCR for Herd-Level Status:

Pooled PCR can be a cost-effective alternative, especially in larger herds or when a low prevalence is suspected. However, pooling samples reduces sensitivity.

- **Pooling 5 fecal samples:**
 - Sensitivity ~67% (McKenna, 2018)
 - Sensitivity improves with more positives per pool:
 - 1 positive animal: ~62%
 - 2 positive animals: ~86%
 - 5 positive animals: ~99%
 - Recommendation: **Pool samples from animals of similar age** to increase the likelihood of capturing high shedders (Kalis, 2000).
- **Pooling 10 samples:**
 - Newer evidence (Ly, 2021) shows **comparable sensitivity** to pools of 5, with significant cost savings (see figure).

Note: Pooling is a herd-level screening tool and is not suitable for certifying individual animals as negative.

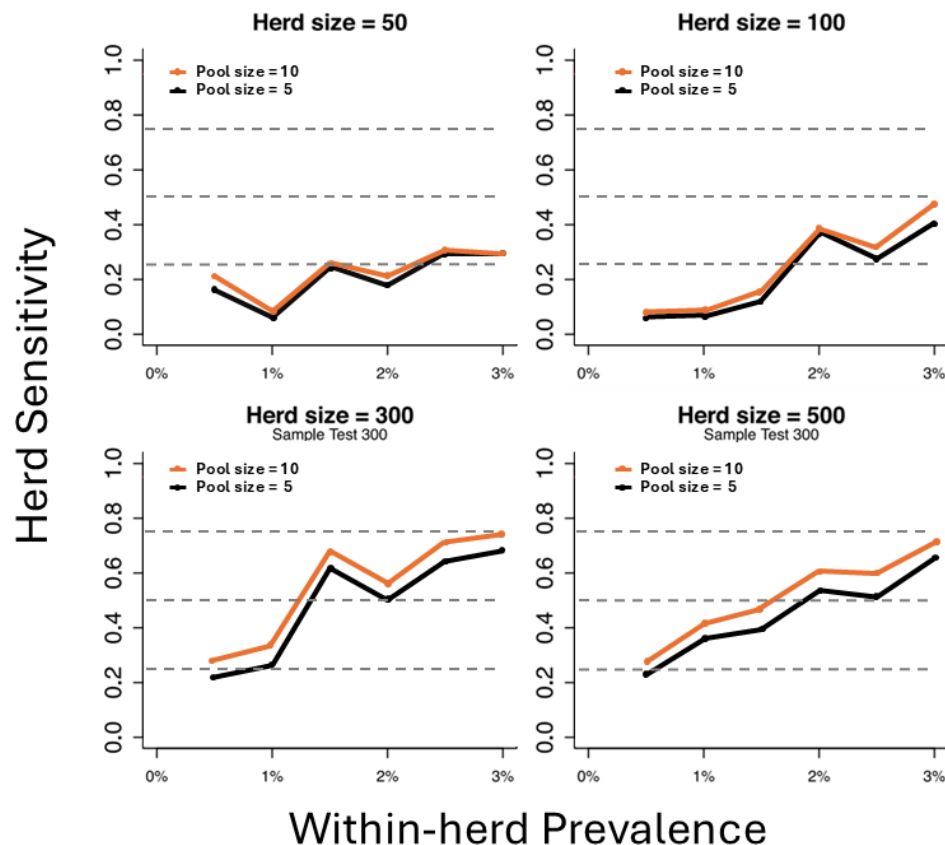


Figure 1. Herd-sensitivities reported by Ly *et al.*, 2021(results derived from Fig. 2), calculated for different pool sizes and low prevalence levels for herds of 50, 100, 300, and 500 cattle.

WSVL Tests for Your Needs (WOAH, 2025)

	Testing Goal (Purpose)					Pre-purchase testing (Seller)	Pre-purchase testing (Buyer)
	Determine if MAP is present in my herd	Estimate within-herd prevalence	Control MAP in an infected herd	Diagnose Johne's in a sick animal			
PCR	+++	+	+	++	+++	+++	
ELISA	+++	+++	+++	+	+++	+++	
Recommended Test(s)	Targeted ELISA or individual PCR (older/thinner cows), Whole herd test (ELISA or pooled PCR), Environmental PCR (not recognized by USDA for Johne's Test Negative Status Program in beef herds).	ELISA on older animals (≥ 2yr), Pooled PCR (if low prevalence suspected)	ELISA or individual PCR on older animals (≥ 2yr), PCR on clinically ill animals	Individual fecal PCR, Necropsy with histopathology and PCR on content from ileum (+/- mesenteric lymph nodes)	Often ELISA on the individual animal(s) being sold.	Ideally, negative whole-herd PCR testing on the source herd. Individual PCR on the animal of interest. If only individual testing is feasible, be aware of the limitations of a single test, especially in young animals.	
Considerations	Consider herd size, cost, and desired level of certainty. In low prevalence herds, PCR may be a more sensitive initial screening tool.	ELISA provides a good estimate of the proportion of infected adult animals, keeping in mind the limitations related to sensitivity and stage of infection.	Regular testing and culling of positive animals are crucial. Consider using fecal PCR to confirm positive ELISA results for a more definitive status.	Necropsy can rule out other causes of disease. PCR on manure can provide a rapid diagnosis, especially in herds with a known history.	Focuses on the animal being sold; lower sensitivity may miss early infections. A negative result does not guarantee the animal is not infected.	Provides a higher level of assurance regarding the infection status of the source herd. Individual tests have limited ability to detect early infections. Knowing Johne's status of the source herd is the most reliable information. Consider repeated negative testing of purchased animals over time.	

Key: +++ = recommended for this purpose; ++ recommended but has limitations; + = suitable in very limited circumstances.

Important Considerations for Testing

- **Test Adult Animals (≥ 18 months):** Diagnostic tests are most reliable in adult cattle, sheep and goats, as they are more likely to be shedding MAP or have detectable antibodies.
- **Customized Testing Strategies:** Tailor your testing approach to align with your herd's specific goals, risk factors, and management conditions.
- **Proper Sample Handling:** Follow lab guidelines for sample collection and packaging to prevent contamination and ensure test accuracy.

Control and Prevention Strategies: Integrating Diagnostics

Diagnostic testing is a cornerstone of Johne's disease control and prevention. Since no effective treatment exists, identifying infected animals is key to limiting the spread of MAP – particularly to young, susceptible calves, lambs, and kids. The timeframe for achieving control depends on herd prevalence and the chosen strategy.

Key Control Measures:

1. **Test and Cull Infected Animals:** Conduct annual whole-herd or targeted testing (via ELISA or PCR) of adult animals to identify and remove MAP shedders. Shedding often increases during stress (e.g. calving/lambing, extreme weather, feed changes, illness). Testing **before the calving/lambing season** is strongly recommended.
2. **Manage Offspring of Positive Animals:** Offspring of MAP-positive dams are at higher risk of infection. Consider culling them or, if retained, **do not use them as replacements**.
3. **Implement Sound Herd and Environmental Management:**

MAP can survive in the environment for up to a year. To limit environmental transmission:

- **Protect feed and water sources** from manure contamination. Fence off contaminated ponds and provide clean drinking water.
- **Maintain clean calving areas** and avoid overcrowding. Move pregnant cows to fresh calving pastures and promptly segregate calves by age ([Sandhills Calving System principles](#), and strategies to prevent neonatal calf diarrhea [Pence et al, 2001; Radostits et al 1983; Thomson, 1997]). Similar management strategies are effective for ewes and does.

- **Manage traffic areas** by placing mineral feeders and hay away from high-traffic calf zones to reduce manure buildup.
- **Rotate pastures** to help reduce MAP persistence.

4. Biosecurity – Prevent Disease Introduction

- Source animals from herds with documented **negative Johne's status** based on whole-herd testing.
- Avoid introducing manure, colostrum, or milk from unknown-status herds.
- Quarantine new additions and consider **repeated testing over time**, especially if sourced from high-risk herds.
- **Know the status of the source herd** – this is more reliable than testing a single animal.

Prevention: The Most Cost-Effective Approach

Preventing the introduction of Johne's disease is far more economical than managing an existing infection. When purchasing animals:

- Prioritize those from test-negative herds with a documented health history.
- Be cautious of animals from herds with **unknown or unverified Johne's status**.

Test Strategy Checklist

To develop a strategic testing plan, consider the following:

- **What is your primary goal?**
(e.g. disease detection, prevalence estimation, control, or verification of status)
- **Which test(s) will be used?**
ELISA, PCR, or a combination?
- **Which animals will be tested?**
Individuals, specific risk groups, or the entire herd?
- **When will testing occur?**
Pre-calving/lambing, pre-breeding, or another strategic timepoint?
- **What decisions will be based on the results?**
Culling, segregation, or other management steps?
- **How will test results be integrated with other herd data?**
Use test outcomes in conjunction with health and production records for comprehensive decision-making.

By understanding and applying available diagnostic tools – and integrating them into thoughtful, herd-specific control and prevention programs – veterinarians and producers can work together to **minimize the impact of Johne's disease** on beef cattle and small ruminant operations.

Online resources with additional information

[Disease Alert: Johne's Disease in Livestock - Animal and Plant Health Inspection Service](#)

Johne's disease (pronounced "yo-knees") is an infection of the small intestine that affects cattle, sheep, goats, and other ruminants (hoofed animals).

www.aphis.usda.gov

[Johne's Information Center - University of Wisconsin – Madison](#)

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[Johne's Disease Program - Cornell University College of Veterinary Medicine](#)

Sampling and Testing Options. The NYS Department of Agriculture & Markets provides Johne's testing subsidies for all cattle herds in New York State. The Johne's Program in New York State (NYS) is administered through the NYS Cattle Health Assurance Program (NYSCHAP). An additional testing subsidy is provided to herds enrolled in the NYSCHAP Johne's Module.

www.vet.cornell.edu

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