

# Use of Tests in Control of *M. paratuberculosis*

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# Acknowledgements

 **USDA** Animal Plant Health Inspection Service | Veterinary Services  
Centers for Epidemiology and Animal Health



# How can test results be used in herd control programs?



1. Detecting infected herds / Herd classification
2. Use in known infected herds

# Detection of antibodies to *M. paratuberculosis*



- ELISA – serum and milk samples
  - IDEXX Herdchek, Prionics Parachek
  - Automated high volume system
  - Relatively low laboratory cost
  - Detect antibodies, not fecal shedding



# *M. paratuberculosis* antigen detection

1. Solid media culture systems
  - 8-16 weeks
2. Liquid culture systems
  - a. Trek ESP (Shin, 2000)
    - Heavy shedders = mean 15 days to detect
    - Light shedders = mean 35 days to detect
  - b. BD MGIT 960 (Cernicchiaro, 2006)
    - Heavy shedders = 78% detected by 28 days
    - Light to moderate shedders = 21% detected by 28 days
  - c. Need confirmation step (culture or PCR)
3. Fecal PCR tests (usu. qualitative results)



# Estimated validity of JD diagnostic tests

Wells et al. 2006. *Clinical and Vaccine Immunology* 13:1125.

| Test          | Sensitivity | Specificity |
|---------------|-------------|-------------|
| ELISA A       | 27%         | 94.9%       |
| ELISA B       | 26%         | 99.8%       |
| Fecal culture | 75%         | 99.8%       |
| Fecal PCR     | 29%         | 99.3%       |

# How to identify infected herds?

## US Voluntary JD Herd Status Program

| Level | Herd test     | Cows tested    | Prob. free from infection |
|-------|---------------|----------------|---------------------------|
| 0     | None          |                | 70%                       |
| 1     | Serum ELISA   | Random 30 cows | 85%                       |
| 2     | Serum ELISA   | All cows       | 95%                       |
| 3     | Fecal culture | All cows       | 98%                       |
| 4     | Serum ELISA   | All cows       | 99%                       |

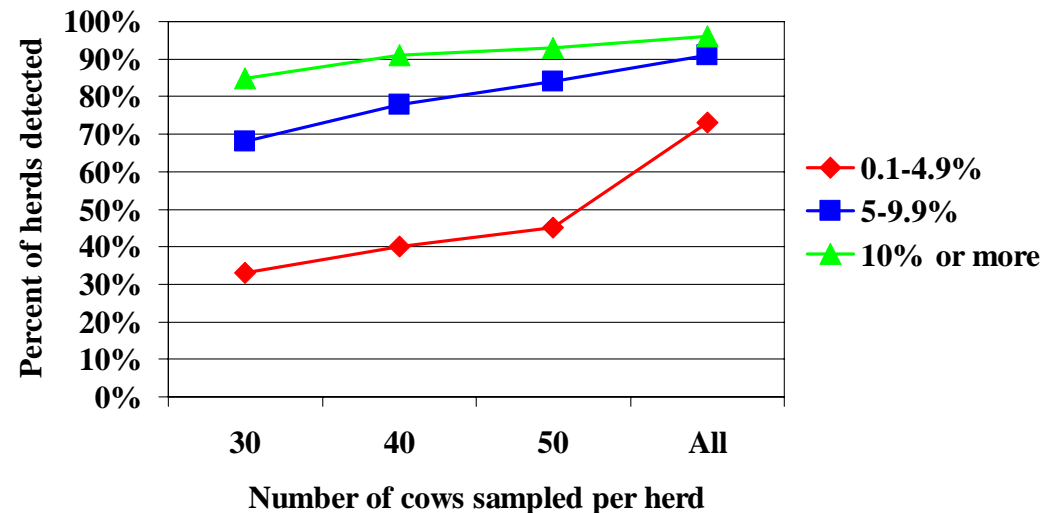
Testing 2+ lactation cows only (and bulls 2+ years) with fecal culture follow-up of test-positive cattle

Standard Track

# Knowns for *M. paratuberculosis* detection

Detection of *M. paratuberculosis* using individual cow testing is costly and imperfect

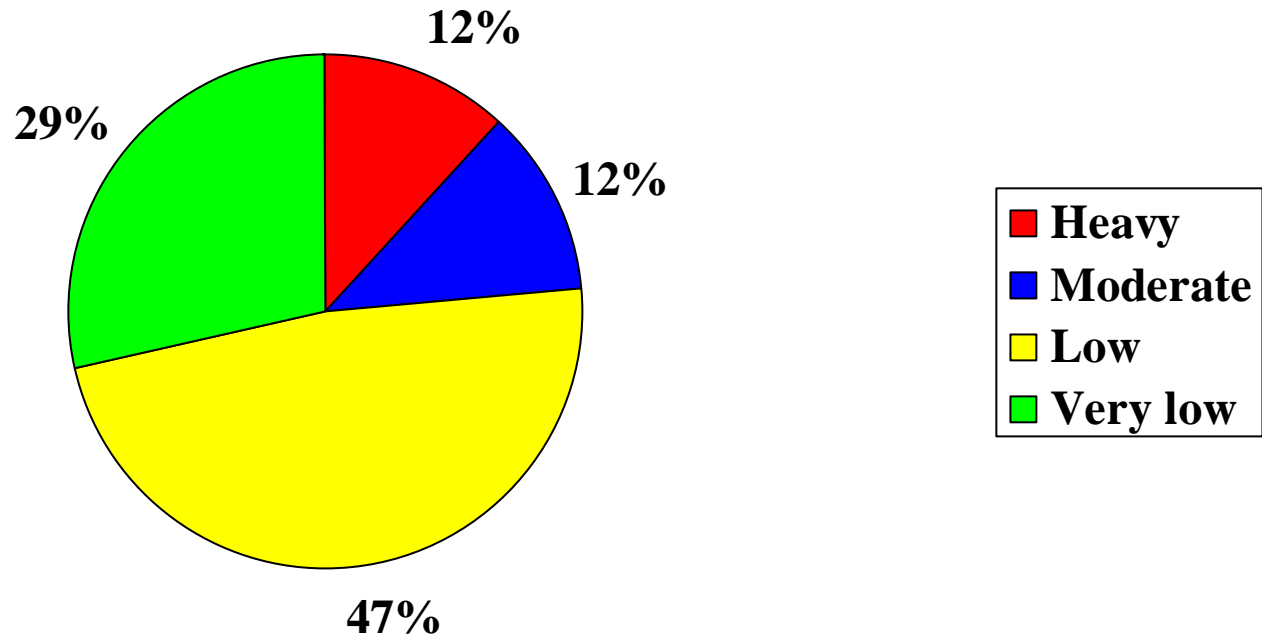
Percent of infected herds detected using Test Negative Program testing (Wells et al, JAVMA, 2002)



**Most low prevalence herds not detected by ELISA/culture follow-up protocols**

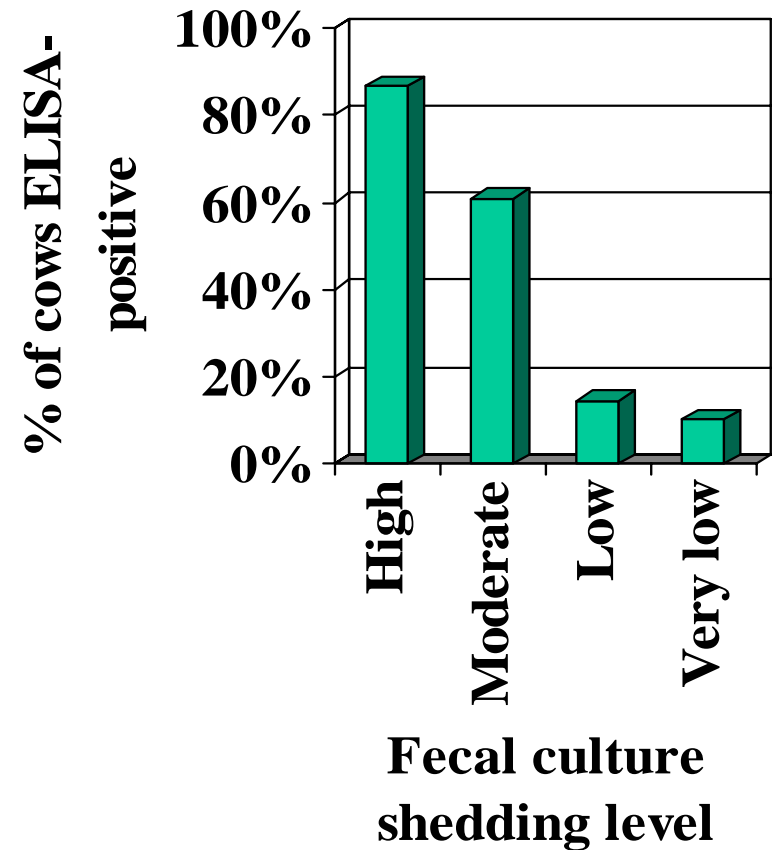
# Percentage of positive *M. paratuberculosis* fecal cultures by shedding level

NAHMS Dairy 2002 Study



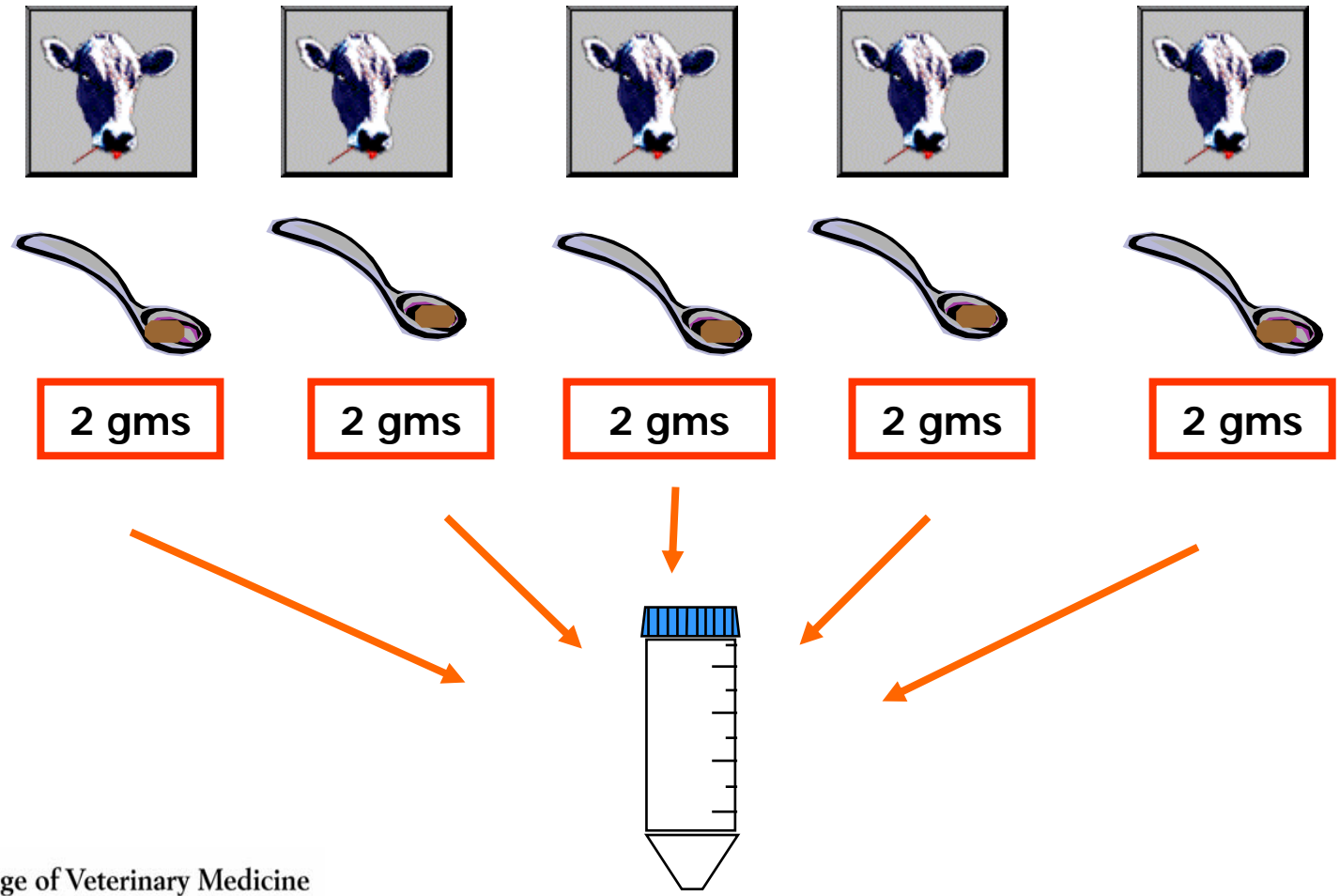
# Knowns for *M. paratuberculosis* detection

Cows shed variable concentrations of Map in feces, partly based on stage of infection, and those shedding high concentrations are easiest to detect.



NAHMS Dairy 2002 Study

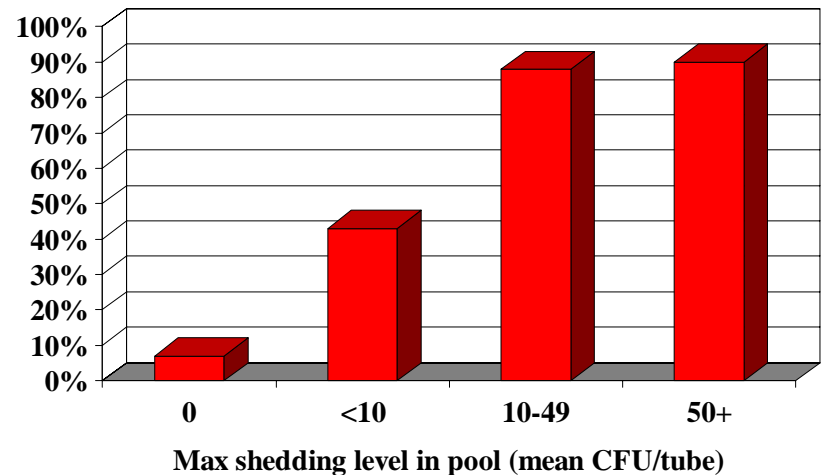
# Use of pooled fecal samples for detection of *M. paratuberculosis*



# Knowns for *M. paratuberculosis* detection

Bacterial culture of pooled samples effective in detection of pools with cows shedding high concentrations of Map in feces.

Percent of fecal pools culture-positive (Wells et al, JAVMA, 2003)

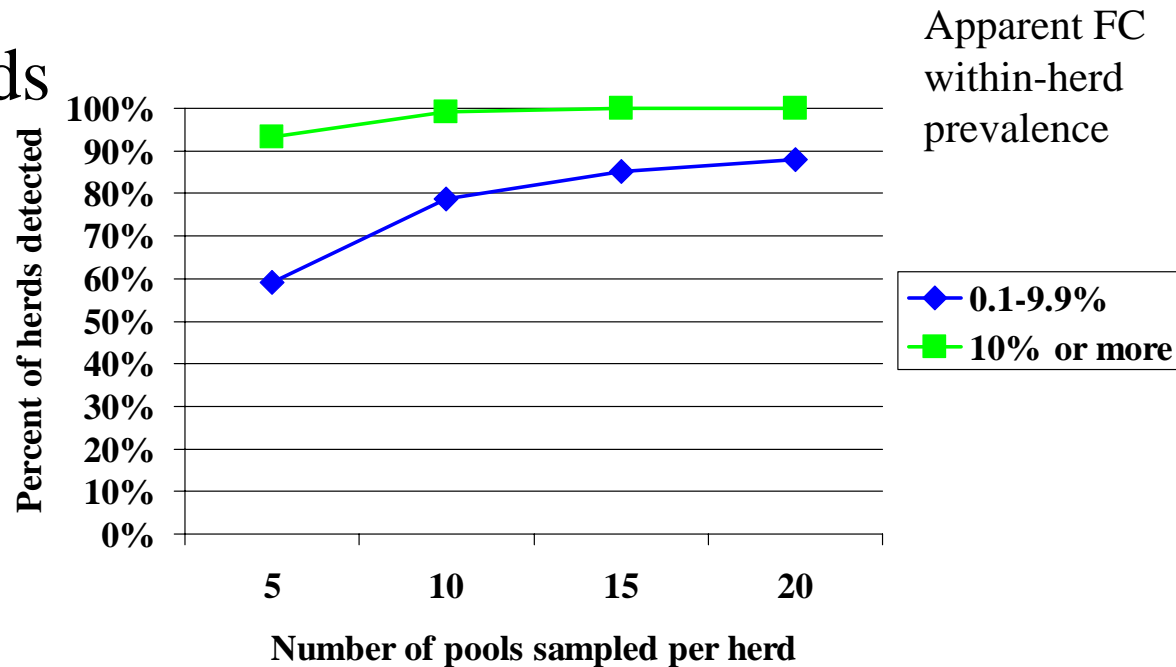


**Detected 90% of pools with at least one cow shedding moderate to high levels**

# Knowns for *M. paratuberculosis* detection

Culture of pools of fecal samples from individual cows detects most herds with culture-positive cows (Kalis, 1999)

Testing 10 pools per herd detected 100% of high prevalence herds and 79% of low prevalence herds (Wells et al, JAVMA, 2003)



(5 cows per pool)

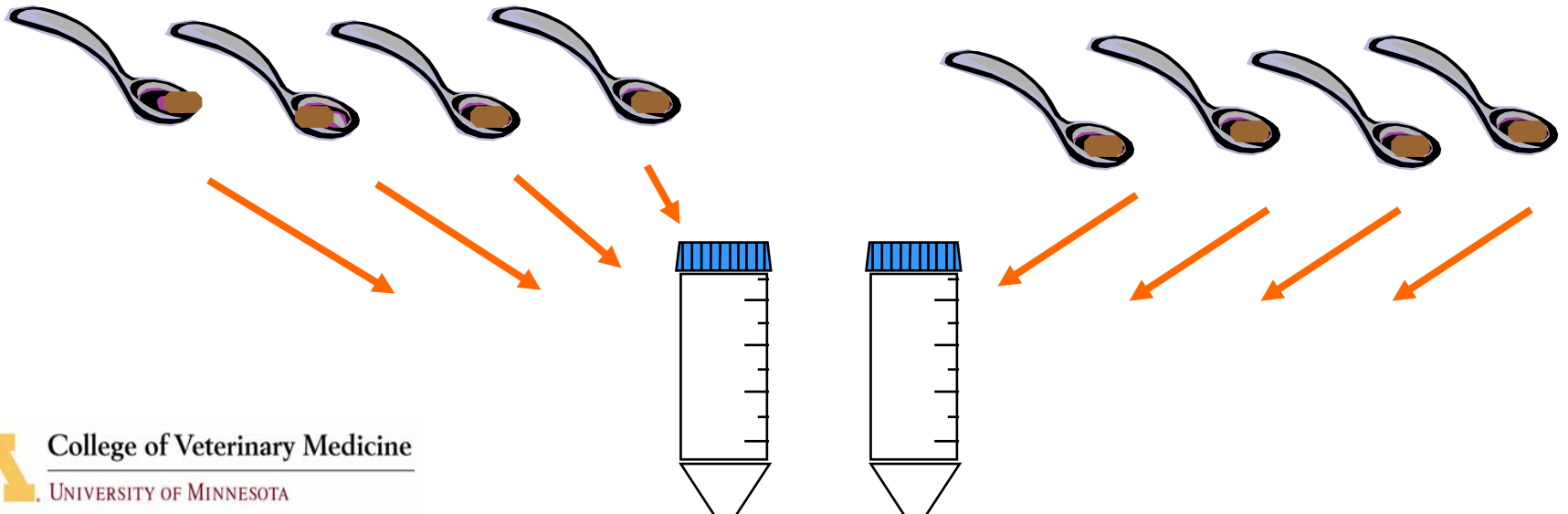
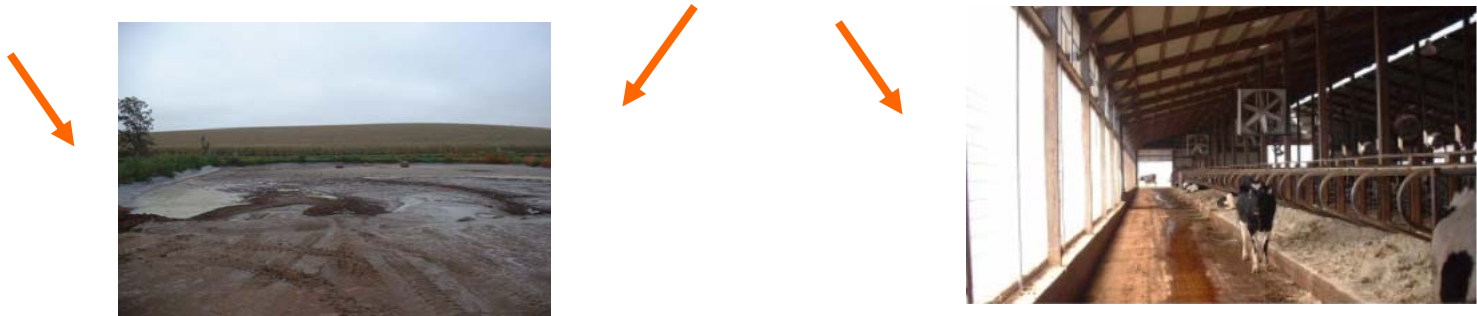
# Knowns for *M. paratuberculosis* detection

*M. paratuberculosis* is capable of persisting in environment of infected cattle herds for long periods

- Cool temperatures (Jorgenson, 1977)
- Shaded environment up to 55 weeks in soil (Whittington, 2004)



# Use of environmental fecal samples for detection of *M. paratuberculosis*



# Environmental sample

## *M. paratuberculosis* culture results

### NAHMS Dairy 2002 Study

| Source          | Samples | % positive |
|-----------------|---------|------------|
| Parlor exitway  | 65      | 52.3%      |
| Holding pen     | 53      | 49.1%      |
| Common alleyway | 127     | 48.0%      |
| Lagoon          | 38      | 47.4%      |
| Manure spreader | 26      | 42.3%      |
| Manure pit      | 41      | 41.5%      |
| Other           | 123     | 34.1%      |
| Total           | 483     | 44.7%      |

70% of herds had at least 1 positive sample

# Model to evaluate testing strategies for detection of Johne's disease in midwestern US dairy herds

Tavornpanich et al. 2007. PVM.

- Stochastic simulation model to compare herd sensitivity of testing strategies for detection of *M. paratuberculosis* in midwestern US dairies with no previous testing or culling for JD.
  - ELISA serologic testing by 2 different assays
  - ELISA testing with follow-up fecal culture
  - Individual fecal culture
  - Pooled fecal culture
  - Culture of environmental samples

# Evaluation of testing strategies for detection of paratuberculosis in midwestern US dairy herds

Tavornpanich et al, 2007, PVM

## Summary

1. Herd sensitivity associated with within-herd prevalence, level of Map organisms shed in feces, and number of samples tested.
2. ELISA alone was sensitive and low cost testing method, but without confirmatory testing in non-infected herds resulted in herd specificity problems.
3. Among testing methods with 100% herd specificity, environmental culture was most cost-effective method followed by pooled fecal culture.



# Environmental Fecal Samples tested for Level 1 of Test Negative Program

- 6 samples tested using fecal culture
- Samples collected to represent the adult milk cow herd (all groups and pens)
- Samples collected from cow alleyways and manure build-up areas
- Each sample tested to represent at least 6 samples collected from area
- Samples collected by veterinarians after training

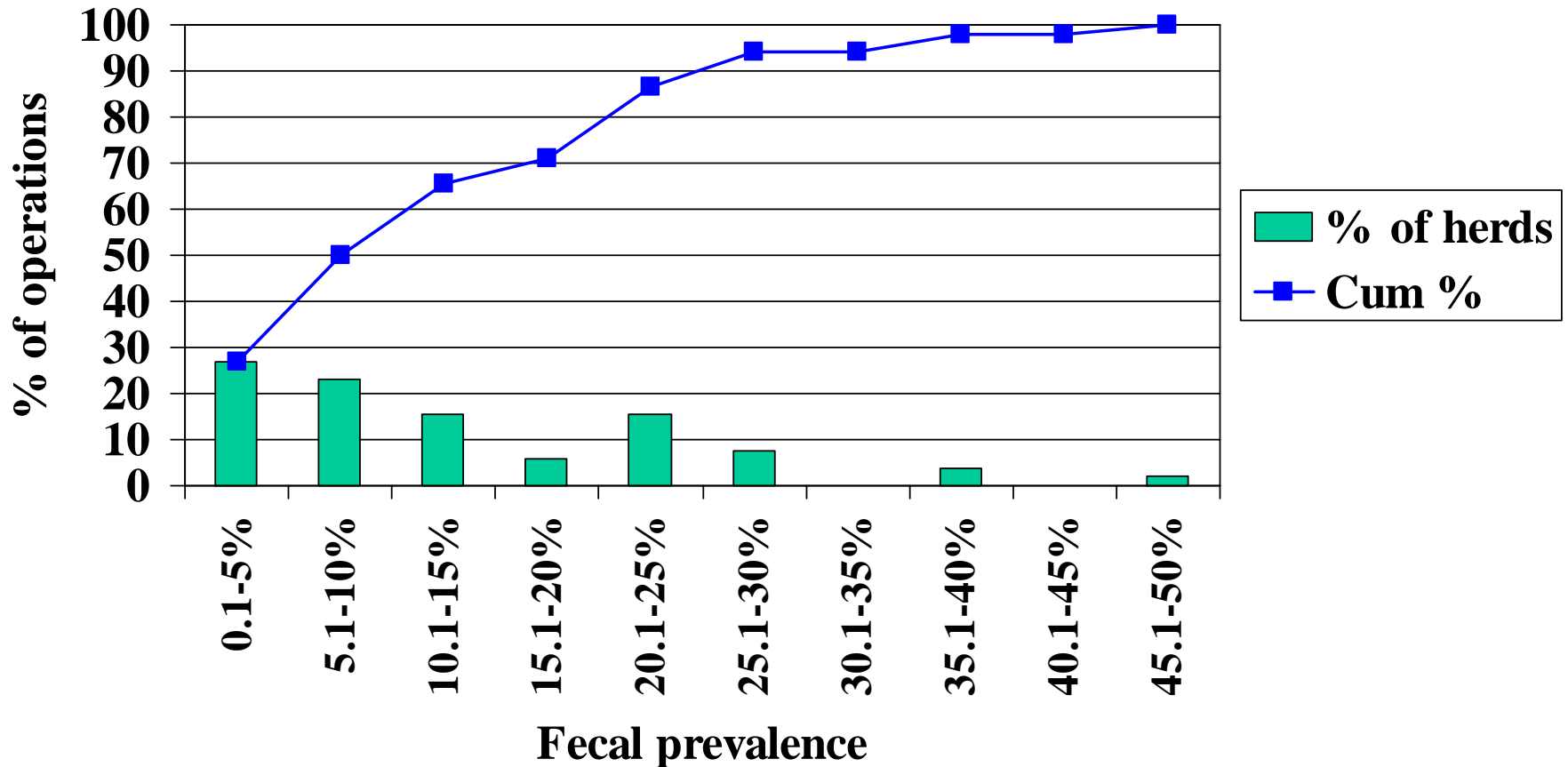
# How can test results be used in herd control programs?



1. Detecting infected herds / Herd classification
2. Use in known infected herds

# Apparent within-herd *M. paratuberculosis* prevalence in test-positive US dairy herds

NAHMS Dairy 2002 Study



# Critical herd management practices for on-farm control of Johne's Disease

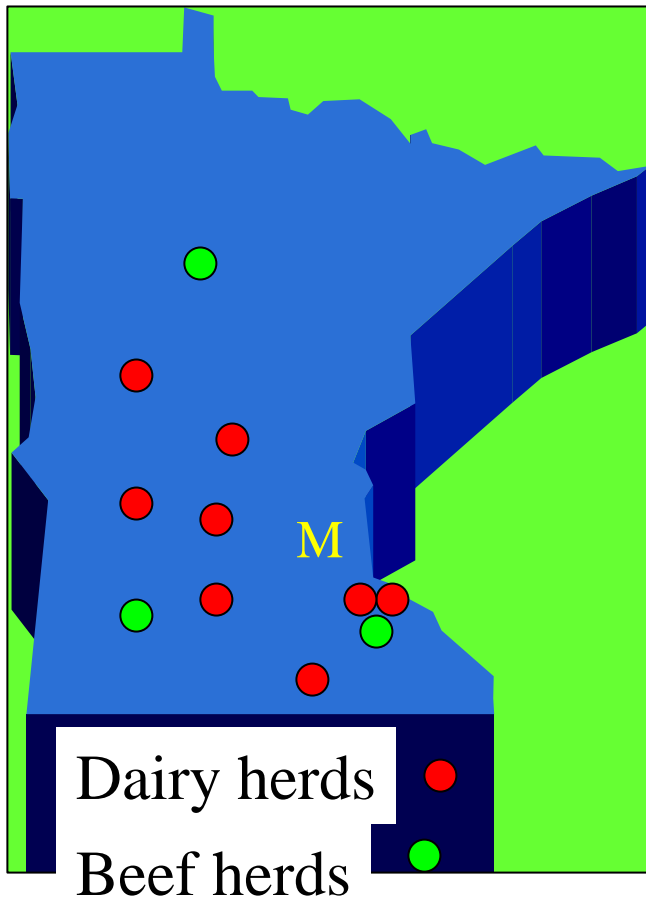
1. Remove all cows with clinical Johne's disease (or high positive test results) to slaughter ASAP.
2. Ensure all cows calve in clean dry calving area; remove calves ASAP after birth.
3. Feed colostrum from dam to calf ASAP after birth.
4. Feed milk replacer to replacement heifers
5. Raise replacement heifers in separate location without contact with older cattle or their manure
6. Use low risk herd replacements

**Maternity  
pen mgt**

**Feeding  
practices**

**Segregated  
heifer  
rearing**

# Minnesota Demonstration JD Control Herds



- 6 dairy herds started in 2000 with onsite heifer rearing
- 3 beef herds started in 2000
- Management changes employed to reduce transmission of JD
- Annual testing of adult cattle using serum ELISA and fecal culture
- Monitor changes through time:
  1. ELISA Prevalence
  2. Culture Prevalence
  3. JD clinical disease

# Conclusions from MN JD Demonstration Herd Control Program

1. Reduction in new infections (seroconversion and fecal shedding)
2. Largest effect on management of cattle up to one year of age
3. No eradication of the disease

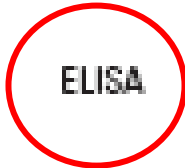


**Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States**

Michael T. Collins, DVM, PhD, DACVM; Ian A. Gardner, BVSc, MPVM, PhD;  
 Franklyn B. Garry, DVM, MS, DACVIM; Allen J. Roussel, DVM, MS, DACVIM;  
 Scott J. Wells, DVM, PhD, DACVPM

Table 3—Recommended test regimen for the detection of paratuberculosis in cattle

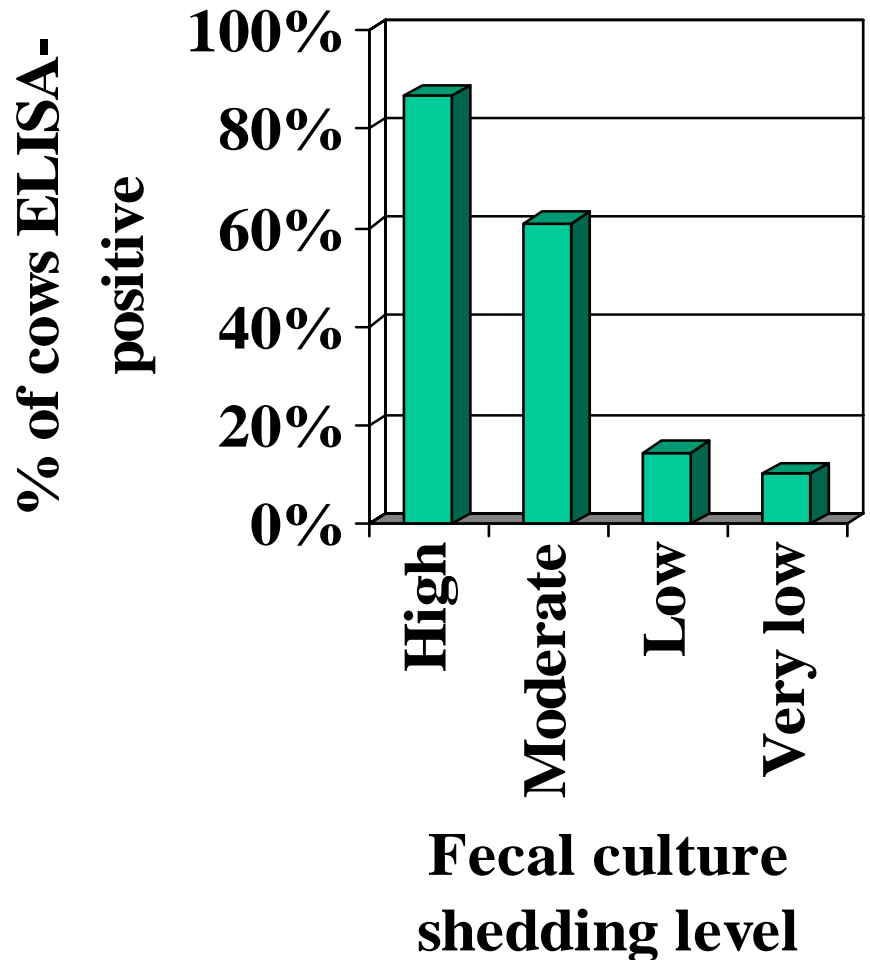
| Testing purpose  | Dairy                                   |   |
|--|---|---|
|  | Commercial                              | Seedstock                               |
| Herd classification (infected or not infected)   | Bacterial culture by ENV-HEY or ENV-LIQ | Bacterial culture by ENV-HEY or ENV-LIQ |
| Precise estimation of within-herd prevalence   | NR                                      | NR                                      |
| Control disease in herd with known infection, high prevalence (> 10% positive results on ELISA), and clinical disease, or owner is concerned | ELISA                                   | Bacterial culture by IND-HEY or IND-LIQ |



# Knowns for *M. paratuberculosis* detection

Cows shed variable concentrations of Map in feces, partly based on stage of infection, and

those shedding high concentrations are easiest to detect.



# Specificity and Sensitivity of Antibody Detection Tests for JD

Collins et al, Clin Diagn Lab Immun, 2005

| Test      | Sample | Spec  | Sens  |
|-----------|--------|-------|-------|
| IDEXX     | Serum  | 95.3% | 28.9% |
| CSL       | Serum  | 99.7% | 28.4% |
| Pourquier | Serum  | 100%  | 27.0% |
| Synbiotic | Serum  | 84.9% | 45.5% |
| Antel-Bio | Milk   | 99.7% | 28.9% |

Test parameters relative to results from fecal culture

# Comparison of milk and serum ELISA for detection of *M. paratuberculosis* in dairy cattle

Lombard et al. 2006. JVDL 18:448

| Fecal culture shedding level | Serum ELISA | Milk ELISA |
|------------------------------|-------------|------------|
| Heavy                        | 92%         | 77%        |
| Moderate                     | 50%         | 54%        |
| Low                          | 10%         | 11%        |
| Very low                     | 15%         | 6%         |
| Negative                     | 2%          | 2%         |

# Decision analysis model for paratuberculosis control in commercial dairy herds

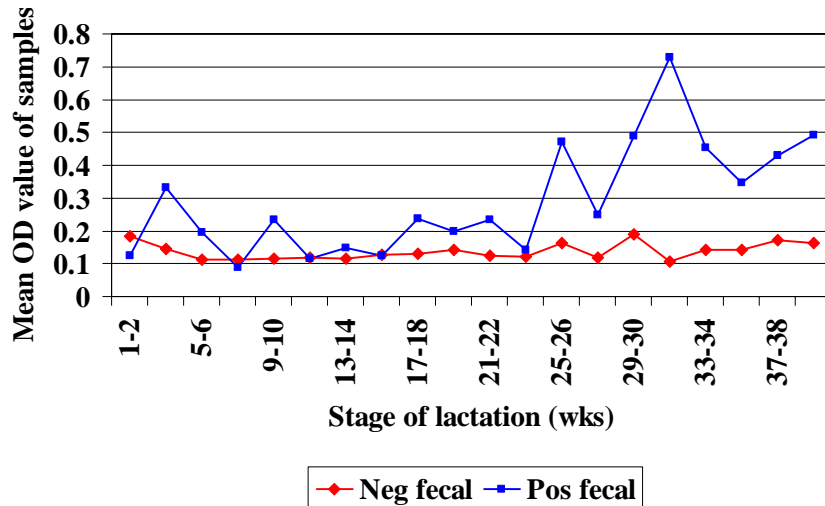
Dorshorst et al. 2006. *Prev Vet Med.* 75:92.

- The economically favorable paratuberculosis control program ... includes managing the herd at HH-1 and regular herd testing with the milk ELISA.
- The model found that commercially available milk ELISA for individual cow milk samples was most often the best test (highest cost–benefit).

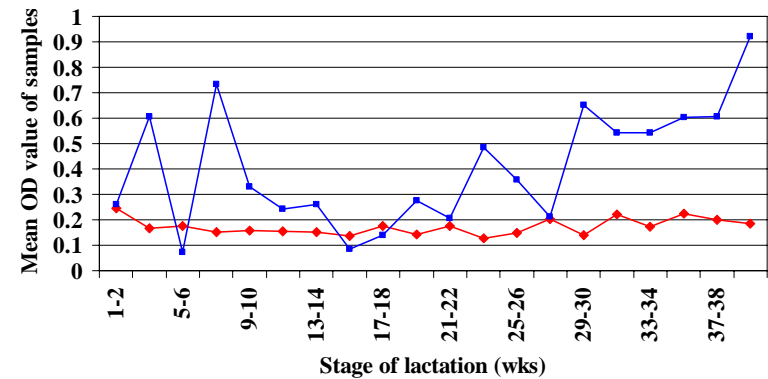
# Mean ELISA values for milk samples from cows by stage of lactation

Nielsen et al, J Dairy Sci, 2002

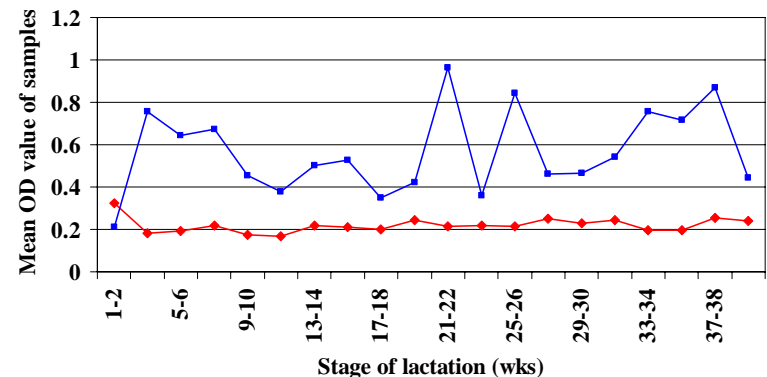
## 1st lactation



## 2nd lactation

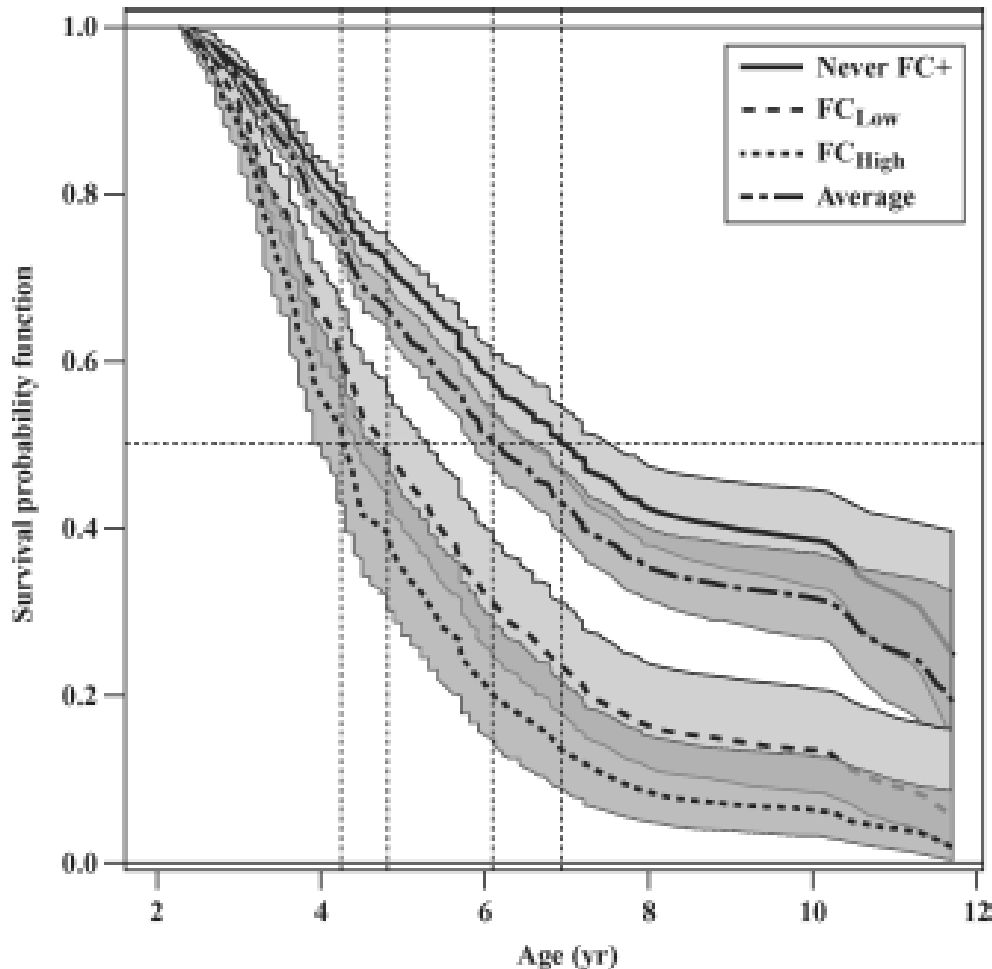


## 3+ lactations



# Survival probability plot of age at occurrence of *M. paratuberculosis* antibodies in cows

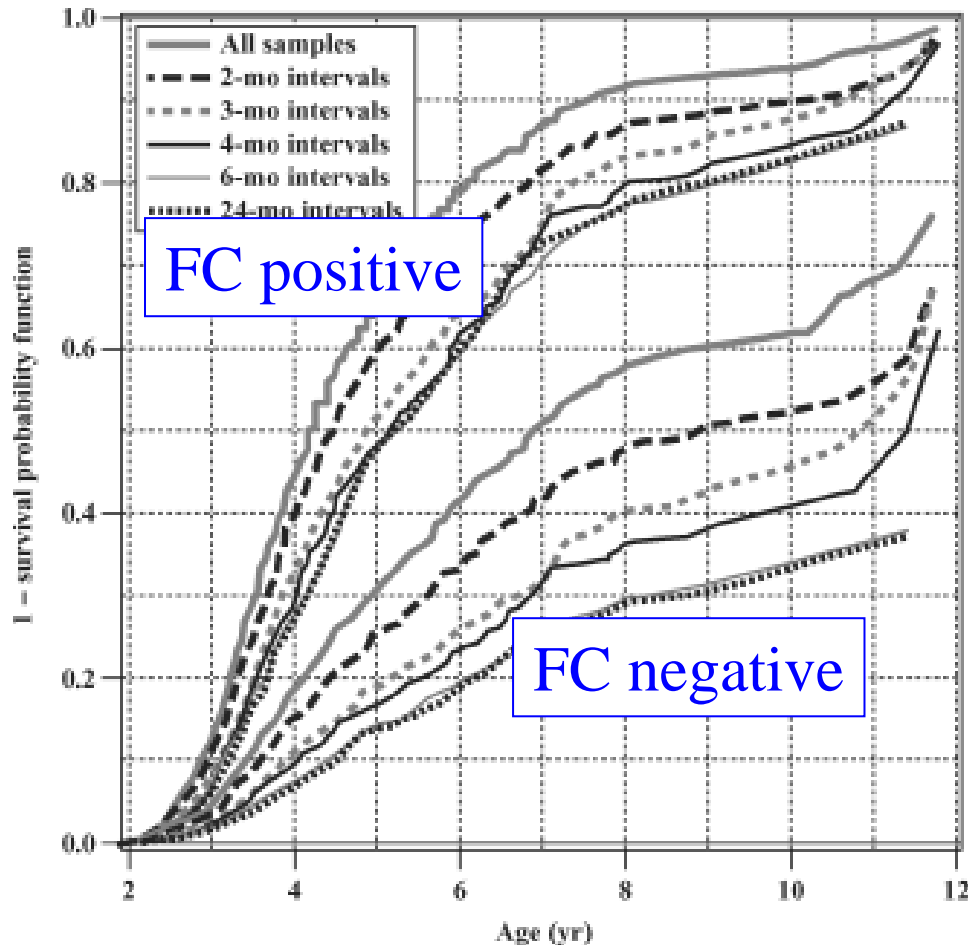
Nielsen and Ersboll, 2006, JDS 89:4557.



- Almost all cows shedding high levels of *M. paratuberculosis* showed antibody in lifetime
- Highest risk of testing antibody-positive from 2.5-4.5 yrs of age

# Effect of sampling interval on probability of testing *M. paratuberculosis*-positive with milk ELISA

Nielsen and Ersboll, 2006, JDS 89:4557.



- Monthly testing could increase sensitivity of test-positive up to 4 yrs of age
- Best time to test cows from day 7 to day 350 of lactation

# Danish JD Control Program

Nielsen et al. 2006. Proc. 1<sup>st</sup> Paratuberculosis Forum

- Voluntary participation
- Goals
  - Provide tools to farmer to control JD
  - Reduce prevalence of JD in country
- Implemented in 2006 by Danish Cattle Federation
  - Danish Dairy Board
  - Danish Farmers Unions
  - Danish Livestock and Meat Board (trade cooperative)
  - Dansire (Breeding cooperative)

# Danish JD Control Program

Nielsen et al. 2006. Proc. 1<sup>st</sup> Paratuberculosis Forum

- Control program includes regular testing of all lactating cows, risk assessment, herd plans, and risk communication
- Test dairy herds 4 times per year with milk ELISA (3 times per cow per year)
  - Expect some false positive and false negative test results

| Cows   | Test | Interpretation                                      |
|--------|------|---|
| Green  | Neg  | Low risk – colostrum donors                         |
| Yellow | Pos  | Calve again but with precautions, not use colostrum |
| Red    | Pos  | High risk – not calve again, may cull               |

Use at least 2 tests for cow decisions

# *M. paratuberculosis* Milk ELISA

## Strengths

- Comparable to serum ELISA
  - detects most high fecal shedders
  - Automated low cost test
- Lower collection cost than serum ELISA
- Easy use of data since linked to DHIA database

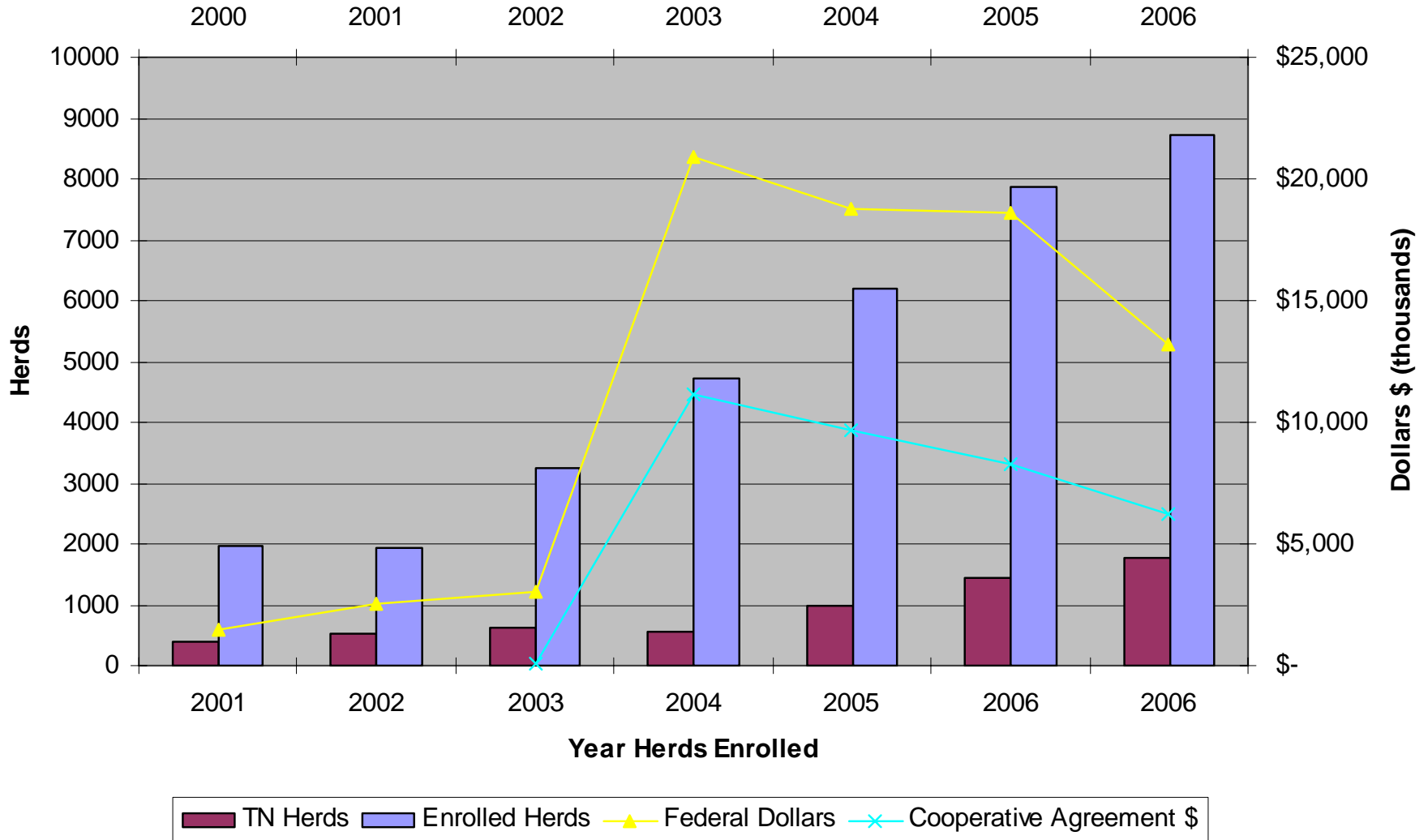
## Limitations

- Low sensitivity
- Imperfect specificity
- Potential variation in milk ELISAs?
- No NVSL check test to date

# What is the future of US JD control programs?

# Enrolled Herds versus Federal Dollars Invested

FY of Cooperative Agreement Dollars



# Summary



- Johne's disease can be controlled on cattle herds
- Use of appropriate tests
  - Herd classification
  - Herd control
- Milk ELISA is important component of future JD Control Program
  - Need for national check test