CORRELATION OF DIAGNOSTIC METHODS AND DISEASE CONTROL PRACTICES WITH REDUCTION IN PREVALENCE OF *M. PARATUBERCULOSIS* IN DAIRY CATTLE

Lawrence J. Hutchinson, Arian Zarkower
Department of Veterinary Science
The Pennsylvania State University
University Park, PA 16802-3500 USA
Robert H. Whitlock, Raymond W. Sweeney,
David T. Galligan, Pamela A. Spencer
New Bolton Center
School of Veterinary Medicine
University of Pennsylvania
Kennett Square, PA 19348-1692 USA

Introduction

Mycobacterium paratuberculosis, Johne's disease (JD), is a chronic, incurable enteropathy affecting cattle and other ruminants. Both diagnostic and control measures have been inadequate to enable early detection and elimination of the infection from cattle herds.

This study attempts to develop and evaluate diagnostic methods and to correlate management and disease control practices with reduction in prevalence of JD-infected animals from known JD-positive herds.

Methods

Twenty Pennsylvania dairy herds with 5% or greater prevalence of JD culture positive cows were selected. Herds were paired by herd size and initial prevalence of JD. One herd of each pair was randomly selected for annual testing of adult cows by fecal culture; the other herd of each pair was tested twice a year for three years with a more sensitive modification of the fecal culture, youngstock were cultured and management/control measures were recommended. In both groups of herds, an annual survey of management practices was conducted.

Standard Program	Intensive Program
culture cows only	culture cows and youngstock
culture 1x/year, sedimentation	culture 1x/year, sedimentation
	culture 2x/year, centrifugation
	management changes including:
	• youngstock separation
	• sanitation

Accepted methods of fecal culture (1) and enzyme-linked immunosorbent assay (2) were evaluated for detection of JD. Modification of the fecal culture method, including varying quantities of inoculum and centrifugation prior to incubation (3) was compared to the standard sedimentation method. A dot immunoassay (DIA) was developed (4) and compared with ELISA results on samples from culture positive and culture negative animals. The DIA and ELISA tests were conducted both with and without preabsorption of sera with *M. phlei*.

A recently available gene probe[a] was evaluated using fecal specimens that were culture positive or negative.

[a] IDEXX Corp., Portland, Maine, USA.

Fourteen of the twenty test herds were participating in a Dairy Herd Improvement Association testing program. Production and other indices for JD-positive and JD-negative cows in these herds were compared.

Results

The sedimentation method of fecal sample preparation for culture was compared with split samples which were centrifuged prior to culturing. Three sample sizes, 0.5, 2.0, and 5.0 gm, were compared for each method.

Table 1. Comparison of culture methods: sedimentation vs. centrifugation prior to fecal culture.

Fecal Culture of 56 Cows		
Sample Size	No. Positive	
	Sedimentation	Centrifugation
0.5 gm	8	18
2.0 gm	18	26
5.0 gm	21	29

At each sample size, more positives were detected by centrifugation prior to culturing. Centrifugation prior to culturing was used in all subsequent culturing during this study except for standard program herds which tested at a state diagnostic laboratory using sedimentation prior to culture.

Sera for fecal culture positive and negative cows were tested using ELISA and DIA methods.

Table 2. Comparison of ELISA and DIA with fecal culture.

Fecal culture	N	Positive ELISA		Positive DIA	
		Unabsorbed	Absorbed*	Unabsorbed	Absorbed*
Positive	28	18	14	24	19
Negative	66	10	2	20	1
Total	94	28	16	44	20
Sensitivity		64.3%	50.0%	85.7%	67.9%
Specificity		84.8%	97.0%	70.0%	98.5%

*Sera absorbed with *M. phlei* prior to testing.

Both tests provided acceptable performance for use as a herd screening tool. The level of bacterial shedding influenced the probability of detection by either serologic method. Fecal samples that were positive using centrifugation prior to culturing were categorized as low level (< 10 colonies per 4 tubes cultured, intermediate level (10 to 100 colonies per 4 tubes cultured), or too-numerous-to-count (TNTC; greater than 100 colonies). Both ELISA and DIA failed to classify as positive some sera from cows testing as low level culture positives. (5)

Absorption of sera with *M. phlei* prior to testing, reduced the sensitivity and increased the specificity of both ELISA and DIA tests. (5)

Table 3. The effect of level of bacterial shedding on the sensitivity of serological assays (absorbed DIA and ELISA).

Level of bacterial shedding	No. of sera tested	No. of ELISA +	(%)	No. of DIA +	(%)
TNTC	8	8	(100)	8	(100)
Intermediate (> 10 colonies)	3	2	(67)	3	(100)
Low level (< 10 colonies)	17	4	(24)	8	(47)
Total	28	14	(50)	19	(68)

The DNA probe was compared with fecal culture. The probe was 100% specific and detected most heavy shedders but failed to detect low-level shedders.

Table 4. The effect of level of bacterial shedding on the DNA probe assay.

Level of bacterial shedding, centrifugation-culture	No. of fecal samples tested	No. of DNA probe +	(%)
TNTC	8	6	(75)
Intermediate level (> 10 colonies)	3	2	(67)
Low level (< 10 colonies)	17	0	(0)
Total positive	28	8	(29)
Total negative	66	0	(0)

In ten study herds (standard program) all cows were fecal cultured annually using the sedimentation method of sample preparation. In a second group of ten herds (intensive program) the same annual culturing protocol was performed. In addition, this second group of herds was cultured twice a year, using the centrifugation method of sample preparation. All cows, heifers and calves were tested at each sampling in these herds.

In the intensive program group of ten herds, 306 positive samples were detected compared to 74 positives in the standard program samples, although both groups had similar cow numbers and test positives in the

year preceding the study. Similar numbers of positive animals were detected in each group using the standard test.

Table 5. Standard vs. intensive program fecal culture positive animals.

	# Tested cows	# Tested heifers	# Positive standard testing	# Positive intensive testing
Standard program herds	1,497	--	74	--
Intensive program herds	1,395	605	70	306

Most of the seventy sedimentation culture positives from the ten intensive program herds were heavy shedders (TNTC) when split samples were tested using centrifugation. This indicates that sedimentation method may be failing to identify light shedding animals with low colony counts. Both centrifugation and testing more then once a year may facilitate early detection and removal of positive animals.

The majority of culture positive animals were first detected during their first or second lactation. Positives in most study herds were promptly culled. Of cows in the study herds with only one or two lactations, 29.2% were JD culture positive, while 14.0% of cows with three or more lactations were culture positive.

Milk production was slightly lower in JD-positive cows compared to herdmates.

Table 6. Milk production per day in milk of JD positive and JD negative cows.

JD-	22.33 kg
JD+	21.11 kg

Average age at first calving and average calving intervals for JD-negative and JD-positive cows were 2.3 vs. 2.2 years and 397 vs. 401 days.

Based upon lifetime records of 290 JD-positive cows and 1,377 JD-negative herdmates, the risk of leaving the herd was 1.26 greater for JD-positive cows than for JD-negative cows.

Management practices in the 20 study herds were evaluated at the beginning of the study and annually throughout the 3-year duration of the study. In the 10 herds selected for the intensive testing program, identification and correction of management factors suspected of contributing to spread of JD was attempted. Four of these herds housed young calves in close association with adult cows and had poor sanitation in calf areas. Three herds had a calving area that was used for other animals (dry cows, sick cows) and/or had poor calving area sanitation. Three herds failed to promptly cull culture positives and/or animals suspected of having clinical JD. One herd purchased

cattle of unknown health status. Producer implementation of recommended management changes was adequate in eight herds and poor in two herds.

Based in part upon findings from this study, recommendations for a voluntary program for certifying herds as JD-negative were developed. The program, based upon annual ELISA screening tests and fecal culture of all ELISA positives should be available within Pennsylvania starting in 1992.

Control recommendations were developed during the course of this study.

For purposes of Johne's disease control, most herds will be in one of three categories: clean herds, heavily-infected herds, or herds that are lightly infected. The herd approach will be different for each type of herd. Other conditions, such as housing, management, herd size, economics, and herd goals will also influence JD control decisions.

Clean Herd: Test negative or herds with no known or suspected JD animals. The emphasis in this herd should be to prevent introduction of JD into the herd and to establish an early diagnosis if JD is suspected.

Maintain a Closed Herd--JD is most likely to be brought onto a farm by purchase of infected cattle.

Test Purchased Cattle--If a closed herd cannot be maintained, buy from herds with no known history of JD; if possible, test animals prior to purchase; segregate from the rest of the herd and test after arrival.

Establish Biosecurity Measures--Limit access by visitors to your cattle housing and feeding areas; don't share cattle trucks, feeding equipment or other equipment that might expose your herd to manure from other farms.

Consider Herd Certification--Several states are considering or have established voluntary herd certification programs. These are based on regular testing of animals for JD. Certification is granted after several negative, whole herd tests. Certification may offer some advantage in the sale of breeding stock.

Heavily-Infected Herd: A herd that is experiencing clinical JD and/or has more than 5 percent of adult cattle test positive on whole herd JD culture is considered to be heavily infected. In this herd, the first priority is to reduce the level of infection and degree of economic loss.

Control programs should minimize the spread of the JD bacteria from the manure of infected, adult cattle to susceptible youngstock. Spread from infected dam to daughter through the uterus or by colostrum or milk is a less likely, but possible, cause.

Cull Clinical Cows--Any animal with persistent diarrhea and/or unexplained weight loss should be suspected of having JD. Rapid tests, such as AGID, ELISA, or gene probe can be helpful in establishing a diagnosis. Clinical JD cows shed high numbers of organisms in their manure. Isolate them immediately and cull promptly.

Test Herd for JD--Blood test (ELISA) or fecal culture all cows for JD; follow-up ELISA test with fecal culture of all ELISA test-positives; testing of yearling heifers will assist in early diagnosis of positives; test herd at least annually; twice a year will speed up the progress of diagnosis and control; if possible, cull all positives immediately; if this can't be done, cull all clinical JD cows and any heavy shedders on fecal culture right away, then cull light shedders as soon as possible.

Don't Raise Youngstock--If level of infection is high in the herd and complete separation of calves is not possible, consider not starting

any heifer calves until culling and separate calf facilities allow a reasonable chance of raising JD-free youngstock.

Use a Clean Maternity Pen--Since calves are most susceptible to JD infection at birth, they should be born in a maternity pen that is cleaned and disinfected before each use. Other animals should not have access to the maternity pen. A clean paddock, not shared with other animals is an acceptable alternative to a maternity pen.

Separate Calf at Birth--Do not allow natural nursing, but remove the calf as soon as possible after birth to clean housing away from older animals. Feed colostrum by bottle or bucket.

Don't Raise Calves From Clinical JD Cows--There is at least a 25 percent chance that the calf from a cow with clinical signs of JD has acquired the infection before birth.

Keep Heifers Separate From Cows--Youngstock, particularly those one year old or less, should be raised in a separate facility from cows. They should not have access to older cattle, feed or water sources used by older cattle or manure storage/runoff from older cattle.

Cleanliness--Keep housing, feeding and watering areas clean; JD spreads by manure; cleanliness is a major part of controlling this disease.

Consider Vaccination--In herds with a high prevalence of JD, vaccination of young calves with JD vaccine is a way of reducing clinical signs of JD in the herd and minimizing the number of culture positive animals. The vaccine has several disadvantages and will not offer complete protection against infection, but it has proven useful when combined with other control measures.

Lightly-Infected Herds or Herds In the Final Stage of Eradication: If a herd has less than 5 percent infection rate in the cows or has reduced the level of infection by the methods listed above, eradication of JD from the herd is a realistic goal.

Continue Testing--Test all animals down to one year of age at least once a year; twice a year will accelerate the eradication process.

Don't Raise Offspring From Positive Cows--Any positive cow, clinical or not, can transmit JD infection to her fetus through the uterus.

Maintain a Closed Herd--JD-free herds and those in the final stages of eradication have the most to lose by purchase of cattle.

Continue Biosecurity and Sanitation Measures

Johne's disease is an incurable disease. It is also difficult to control and eliminate, but current knowledge and tests do make the process possible. Control measures provide the added benefit of reducing the likelihood of spread of several other diseases. Good management and JD control go hand-in-hand.

References

1. Merkal, R.S., Lyle, P.A.S., Whipple, D.L., Decontamination, Media and Culture Methods for *M. paratuberculosis*. Proc. 86th Ann. Mtg., U.S. Animal Health Assoc., pp. 519-523. 1982. 2. Abbas, B., Riemann, H.P., Lonnerdel, B., Isolation of Specific Peptides From *M. paratuberculosis* Protoplasm and Their Use in an ELISA for the Detection of Paratuberculosis (Johne's Disease) in Cattle. Am. J. Vet. Res. 44:2229-2236. 1983. 3. Whitlock, R.H., Rosenberger, A.E., Fecal Culture Protocol for *M. paratuberculosis*, a Recommended Procedure. Proc. 94th Annual Mtg., U.S. Animal Health Assoc., pp. 280-285. 1990. 4. Tsai, S.J.,

Hutchinson, L.J., Zarkower, A., Comparison of Dot Immunobinding Assay, Enzyme-Linked Immunosorbent Assay and Immunodiffusion for Serodiagnosis of Paratuberculosis. Can. J. Vet. Res 53:405-410. 1989. 5. Reddy, N.D., The Comparison of Dot Immunoassay (DIA), Enzyme-Linked Immunosorbent Assay (ELISA), and the DNA Probe Assay With Fecal Culturing for the Diagnosis of Johne's Disease, Master's Thesis. The Pennsylvania State University. 1991.

Acknowledgements

This project was supported by a research grant from the Pennsylvania Department of Agriculture. The technical assistance of Betsy Rosenberger and Terry Fyack (University of Pennsylvania), Carol Burns and Sharyn Houtz (The Pennsylvania State University) is gratefully acknowledged.

Summary

Johne's disease presents serious challenges for diagnosis and control. Existing and new diagnostic methods were evaluated. Sample centrifugation prior to culturing increased the sensitivity of fecal culture. Both ELISA and dot immunobinding assay, when performed with *M. phlei* preabsorption, proved acceptable tools for screening herd sera. Both of these methods, as well as a gene probe, failed to detect JD positive animals with low colony counts.

Most JD animals were first detectable on fecal culture during their first or second lactation. Milk production per day in milk was reduced by 1.11 kg compared to non-infected herdmates.

Recommended control measures and a voluntary JD certification program are described.

Resumé

La maladie de Johné presente des sérieux défis en ce qui concerne le diagnostique et le control. On a évalué des methodes diagnostiques nouvelles avec d'autres déjà existantes. La centrifugation des echantillons avant les cultures augmente la sensibilité des cultures des selles. On montre que les méthodes d'ELISA et l'essaie d'immunoliaison de point ce sont des instruments acceptables pour le dépistage des serums de troupeaux lorsqu'elles se font en preabsorbant les echantillons avec *M. phlei*. Ces deux methodes, ainsi que la sonde genetique n'ont pas pu detecter des animaux positifs pour JD avec des bas nombres de colonies.

La plus part des animaux etaient detectables par la culture des selles pendant leur premiere ou deuxieme lactation. La production de lait par jour était reduite de 1.11 kg par raport aux animaux non infectes du même troupeau. On recommende des mesures de control et on décrit un programe volontaire de certification de JD.

Resumen

La enfermedad de Johne presenta serios problemas a la hora de diagnosticar y controlar. Hemos evaluado métodos de diagnóstico nuevos y tambien ya conocidos. La centrifugation de las muestras antes del cultivo aumenta la sensibilidad del cultivo fecal. Las pruebas de ELISA y immuno-enlace de punto son instrumentos aceptables para estudiar los seros de rebaños, siempre y cuando se aplique una preabsorbcion de las muestras con *M. phlei*. Ambos métodos, asi como la sonda genética, no detectaron animales positivos para JD con bajo numero de colonias.

La mayoria de animales positivos para JD, se detectaron por primera vez por cultivo fecal durante su primera o segunda lactacion. La produccion de leche por dia se redujo 1.11 kg comparando con animales no infectados del mismo rebaño.

Se describen igualmente, recomendaciones para medidas de control, y un programa voluntario de certification de JD.