Spontaneous Clinical Regression of Johne's Disease in a Holstein Cow: *A Case Report*

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KEY WORDS: Johne's disease, paratuberculosis, cow; self-cure; serology; culture, *Mycobacterium avium* subsp *paratuberculosis*, polymerase chain reaction

ABSTRACT

A case of clinically overt Johne's disease undergoing spontaneous regression occurred in a Holstein cow. Longitudinally measured serologic titers decreased with abating clinical signs of Johne's disease, as did the status of fecal shedding. At necropsy, the animal had no macroscopic changes typical of Johne's disease. Very detailed microscopic examination of multiple target tissues of Johne's disease revealed individual microgranulomas without demonstrable acid-fast bacilli. It is postulated that changes in the cow's environment triggered immune and host-response events responsible for the regression of Johne's disease status from clinical to silent to cure.

INTRODUCTION

Paratuberculosis (Johne's disease) is an insidious, chronic wasting disease affecting ruminants worldwide.[1] The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis*, a facultative intracellular acid-fast bacillus. Neonatal calves are most susceptible to infection by the bacillus, the mode of transmission is thought to be oral-fecal passage. The disease is characterized by a long incubation period, which often lasts several years before clinical signs become apparent. Typically, clinical signs are characterized by protracted diarrhea and weight loss, despite a good appetite. Treatment modalities are not available, and vaccination is not an option to prevent infection. Affected animals generally are culled or die because of severe muscle wasting and protein loss. This report describes the unexpected, uncharacteristic recovery of an animal with clinical signs of Johne's disease to a state of being completely free from diarrhea and exhibiting weight gain and improved body condition.

Animal

A 5.5-year-old Holstein cow from a herd of 500 milking Holsteins developed diarrhea and experienced weight loss and a decline in milk production. The cow had previously (at 4 years of age) been tested by ELISA for *M. avium* subsp *paratuberculosis*, and results were interpreted as suspicious for Johne's disease. When tested at the start of the present study (a year and a half after the initial ELISA), the animal was exhibiting clinical signs of Johne's disease, and the ELISA test was positive, as was an agar gel immunodiffusion test (AGID) (Table 1). The seropositive animal was selected for a study that involved the development of nested polymerase chain reaction (nPCR) to detect infec-

Table 1. Findings of Laboratory Evaluations for a Holstein Cow with Apparent Spontaneous Regression of Clinical Johne's Disease

Date	AGID	ELISA (OD)*	Fecal Culture	nPCR P90,P91† (Blood)	nPCR J1,J2‡ (Blood)	nPCR J1,J2‡ (Milk)
04/15/01	Negative	1.8	ND	ND	ND	ND
09/10/02	Positive	3.2	ND	Negative	Negative	ND
09/24/02	Positive	3.0	ND	Negative	Negative	Positive
11/19/02	Negative	1.8	ND	Positive	Negative	Positive
12/10/02	Negative	1.5	Overgrowth	Negative	Positive	Positive
12/30/02	Negative	2.0	Few colonies	Negative	Positive	Positive
01/21/03	Negative	2.7	Negative	Negative	Positive	Positive
01/28/03	Negative	2.5	ND	Negative	Positive	Positive
02/04/03§	Negative	2.3	Negative	Negative	Positive	Positive

*OD >2.0 is a positive ELISA; <2.0 is suspicious ELISA.
†P90,91 = first primer.
‡J1/J2 = second primer.
§Date of necropsy.
ND = not done.

tion with *M. avium* subsp *paratuberculosis* in blood monocytes and milk macrophages.

The animal was removed from the herd and individually housed at the Veterinary Medical Teaching Hospital at the University of Florida. Food and water were provided ad libitum. Soon after arrival, clinical observation revealed cessation of diarrhea and gain of body condition.

Evaluations

The protocol for the study required serial monitoring of the study animal by ELISA and AGID. These tests were performed seven times after the initial testing (at selection) and again at necropsy, 5 months after the initial evaluation (Table 1).

At the end of the PCR study, the cow was euthanized, and a complete necropsy was performed. A total of seven lymph nodes were randomly selected from the mesenteric chain and 24 sites of the small intestine (from the duodenum to the ileum) for dissection and microscopic evaluation. All tissue sections were stained with hematoxylin–eosin. Lymph node sections were also stained with acid-fast stain.

RESULTS

As the AGID tests went from positive to five sequential negative readings, the ELISA levels fluctuated between suspicious and low positive. On one occasion, fecal culture revealed a small number of colonies that were acid-fast positive and positive by PCR when probed for IS900. When tested by nPCR, the cow had amplicons in blood, milk, or both samples on all six occasions over the 5 months of testing as well as at the necropsy evaluation (Table 1).

At necropsy, the animal weighed 668 kg and was in good nutritional condition. The only gross finding was a perireticular abscess approximately 42 mm in diameter from which *Arcanobacter pyogenes* was isolated. No microscopic changes suggestive of the presence of Johne's disease were observed in the intestines. In a few sections of the mesenteric lymph nodes, occasional small clusters of epithelioid macrophages and an occasional multinucleate inflammatory giant cell were observed in the paracortex (Figure 1). Using special stain and oil immersion, acid-fast bacilli were not detected. Nested PCR analysis of the liver, mesenteric and ileocecal lymph nodes, and spleen was negative for *M. avium* subsp *paratuberculosis*. The final diagnosis confirmed a very minimal presence of Johne's disease, with no acid-fast bacilli present.

DISCUSSION

To date, serologic testing and fecal culture

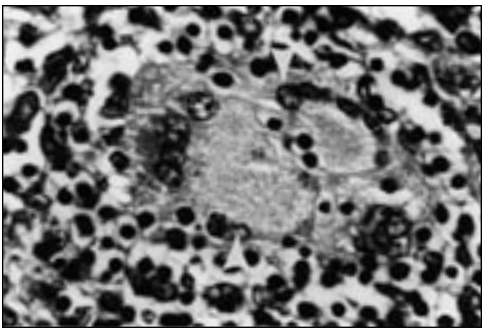

Figure 1. Mesenteric lymph node from cow with spontaneous regression of clinical Johne's disease. A small group of individual Langhans giant cells are embedded within lymphocytes of the paracortical zone (*arrows*). Hematoxylin–eosin, magnification × 100.

combined with culling of animals positive for the organism in either serum or fecal cultures or both have been recommended as management tools to control Johne's disease.[2] These approaches have largely overshadowed efforts at therapy and prevention via vaccination. Occasional anecdotal reports of self-cure are considered an epidemiologic possibility in computer models; however, to the knowledge of the authors, no clinical evidence of regression of the disease in cattle has been reported in the literature.

The most apparent modification of events for this animal was removal from the herd and its competitive environment. The isolation into a less stressful environment resulted in abatement of clinical signs and the decrease of serologic titers, approaching low positive or suspicious levels by ELISA and seronegativity by AGID testing. Fecal culture results suggested a low level of shedding or possible paucibacillary status. The one culture that contained a small number of colonies could not have been a result of passive shedding with organisms from a contaminated environment.[3]

The reversal of the cow's clinical status can be best understood by the paradigm that Johne's disease is imunologically similar to other mycobacterial diseases. It is a spectral disease starting with cell-mediated immunity, followed by nonprotective humoral immunity controlled by T helper cells, switching after a long patent period from Th1 to Th2 subtypes as the animal passes from a silent status to a clinically affected status. The switch is hypothesized to occur at about 12 to 18 months of age in an infected animal, although many infected animals do not show detectable humoral immune responses when they are 2 to 3 years of age.[4] The humoral response is dominated by cytokines, such as tumor necrosis factor-α produced by Th2 cells, whereas Th1 helper cells produce cytokines such as γ-interferon.[5] The key factor responsible for the shift to a humoral immune response remains to be identified. Similarly, the nature of immune factors effective in the reversal of the disease in this animal is not known.

In addition to moving through a spectrum of immunologic changes, infected animals progress through a spectrum of pathologic changes in the target small intestinal tract from early changes that are only detectable as microgranulomas by microscopic examination to grossly visible changes of chronic proliferative enteritis, lymphangitis, and mesenteric lymphadenitis.[6] The latter coincides with the presence of clinical signs and a humoral immunity. During the various stages of infection, there are variations and fluctuations of antibody response and fecal shedding.[7] Silent infection is more difficult to diagnose and is possible only microscopically because lesions are subtle and the presence of the organism is rare.[8] For this approach to be successful, it is necessary to screen many small intestinal sites 7 to 15 meters in length and several mesenteric lymph nodes for microgranulomas, acid-fast bacilli characteristic of *M. avium* subsp *paratuberculosis*, or both. Criteria for such morphologic assessment of early infection have been established in some instances but lack standardization on a worldwide level.[8,9] The cow studied here fit the criteria of early infection after apparent reversal of the clinical disease process. The failure to demon-

strate acid-fast bacilli in tissue sections is probably related to the capability of macrophages and inflammatory giant cells to kill and degrade bacilli, very similar to what takes place in immunocompetent forms of leprosy and tuberculosis.

Direct agent detection through fecal culture is the accepted gold standard for the confirmation of Johne's disease. PCR is considered an alternative to detect antigens that are notorious for requiring laborious specimen handling and an extended time for growth. Presently, fecal PCR is hampered by low sensitivity due to the presence of fecal inhibitory substances.[10]

PCR is a molecular-based technique capable of amplifying genes of mammalian or prokaryotic cells to measurable or visualized amplicons when probed with primers. The nPCR allows further amplification of the signal already amplified by the simple PCR, particularly when the first signals were weak or invisible on agar-gel. PCR assays for IS900 have been reported to detect as low as 10^4 colony-forming units per gram of feces in shedding cows.[10] The aim of the nPCR developed in the authors' laboratory was to detect subclinical infections in the silent phase in blood monocytes, milk macrophages, or both. Testing of 46 cows with subclinical infection revealed only a small number having positive amplicons in blood, milk, or both when animals had a suspicious or negative ELISA.

A case such as this gives cause to reconsider the "test and cull" dogma for management of Johne's disease in the absence of treatment modalities or preventive vaccination. The phenomenon of spontaneous regression of Johne's disease is worthy of more detailed investigation as to the immunologic mechanism and host defense. It is possible these factors may preclude the need to remove individual animals that demonstrate evidence of self-cure from the herd. Perhaps genetic testing could be a consideration in the future to identify the genotype of potentially self-curing cows. Finally, spontaneous regression of Johne's disease should be considered a possibility when managing the epidemiologic pattern of the disease.

ACKNOWLEDGMENTS

The authors thank Mary Taylor and David Armstrong, managers of the dairy herd, and Dr. Carlos Risco, Rural Animal Medicine, College of Veterinary Medicine, University of Florida, for their cooperation and assistance.

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