

Paratuberculosis (Johne's Disease) Update

R.H. Whitlock*, L. T. Hutchinson**, L. T. Glickman*, R. Meinersmann*, C. Rossiter*, J. Bruce*, R. Merkal***, and J. Dick****

*The School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

**Department of Veterinary Science, Penn State University, University Park, Pennsylvania.

***National Animal Disease Laboratory, Mycobacteriology Laboratory, Ames, Iowa.

****Bureau of Animal Industry, Pennsylvania Department of Agriculture, 2301 North Cameron Street, Harrisburg, Pennsylvania.

Introduction

Paratuberculosis (Johne's disease), a chronic infective disease of ruminants, has been recognized in the United States for about 75 years. Johne's disease was named after Dr. P. Johne, who isolated the pathogenic organism in 1895 (Chiodini 1984, Reiman 1983). The disease was named paratuberculosis by Bang in 1906, due to similarities of the etiologic agent to the tuberculosis bacillus. The causative agent is *Mycobacterium paratuberculosis*, a facultative intracellular acid-fast bacterium. Infection with *Mycobacterium paratuberculosis* is difficult to control because of a long incubation time, the absence of clinical signs in the early stages, the lack of effect of antibiotics or other drugs, and the lack of quick reliable methods for diagnosis.

Paratuberculosis, a wasting disease, is characterized by severe diarrhea and rapid weight loss in the later stages of infection. Many infected animals develop mastitis and other secondary diseases. The economic loss due to Johne's may be greater due to these secondary diseases, including infertility, than to the obvious loss associated with muscle wasting and diarrhea. Johne's disease is seen in all ruminants including wild deer, goats, sheep, etc. and occurs worldwide, causing staggering losses to the livestock industry, particularly in third world countries (Sherman 1985).

Usually only one or two cattle exhibit the symptoms of diarrhea and weight loss at any one time. And as these signs can be caused by other diseases, Johne's disease may not be considered, primarily because farmers are unaware of the disease. Frequently the unproductive animals are culled and sold for slaughter without considering Johne's Disease. Then later, more animals in the herd may display the same symptoms. Johne's disease, once in a herd, is hard to eliminate. It has been found that infectious bacilli are shed

by a cow for six to eighteen months prior to the appearance of any symptoms; pastures become contaminated and other cattle, especially calves, are exposed to the bacilli.

Prevalence of Paratuberculosis

Paratuberculosis is widely distributed throughout the world. The disease is usually reported to range from 2-10% (Taylor 1952, Merkel 1973, Rankin 1958). In severely infected herds the prevalence may be up to 25% (Larsen 1968). However, cattle with clinical disease represents only a fraction (15-30%) of the total number of infected animals in the herd (Doyle 1956; Hole 1958; Merkel 1968; and Merkel 1973). Surveys in England in the 1940's and 1950's estimated the prevalence to be 10-30% based on surveys of slaughterhouses (Doyle 1951; Doyle 1956, Hole 1958, Rankin 1958). Recent slaughter surveys in the U.S. in Wisconsin, New England and California report prevalence rates of 10.8, 18 and 9% respectively (Arnoldi 1983, Abbas 1983, and Chiodini 1983). In August 1984, Wisconsin had 1,439 Johne's positive herds out of a total of 69,000 herds. A survey of 12 California herds involving 3,140 cattle found 96 (3.05%) positive for paratuberculosis on fecal culture. Most of positive cattle (76%) shed relatively few organisms (3-9 colonies/slant), whereas 24% shed more than 10 organisms (colonies) per slant. The mean number of clinically affected cattle was 0.5% of all those tested and 15.6% of those animals that were culture positive (Abbas 1983). On a herd basis the prevalence of clinical cases ranged from 0.94% to 13.8% among the infected animals in the herd. Clinical cases were not found in herds shedding less than 10 colonies/slant. In those herds were 3-5% of the animals are culled with confirmed clinical Johne's disease one should anticipate the actual infection rate to exceed 50% (Duncan 1978).

Johne's disease is widespread in the United States where it was reported in 47 states in 1971 (Kopecky). High prevalence of infection was reported for 11 states: California, Florida, Indiana, Iowa, Maryland, Minnesota, Ohio, Oregon, Pennsylvania, Washington, and Wisconsin. A national

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survey for the U.S. has been conducted by the National Animal Disease Center, Ames, Iowa, which should further clarify the prevalence by states. Results of a recent slaughter survey in cows from northeastern states based on 1,224 random samples, estimated the prevalence of paratuberculosis in cull dairy cows from Pennsylvania at 7.2% and 7.3% for the entire northeast region (Whitlock 1984, Whitlock 1985). Slaughter survey prevalence estimates reflect both the incidence rate and duration of the disease and imply that the actual prevalence in the population as a whole is low. Johne's disease probably has a non-random distribution and may be clustered in certain herds or regions of the country. Reliable incidence estimates would require large population surveys and a rapid and accurate means of diagnosis.

Transmission

The natural transmission is believed to occur mainly via the ingestion of feed and water contaminated with infected fecal material. Fecal contamination of the dam's teats may be a major source of infection (Doyle). The ileum, cecal valves and the associated lymph node are the primary sites of infection and proliferation of the organism in the body. The infected sloughed mucosal cells pass out in the stool which serves as a potential source of infection to other animals.

Infected animals of all ages are capable of excreting the organism but the number of organisms tends to increase as infection progresses. Clinical cases shed massive numbers of organisms and are the major source of infection to susceptible animals and environmental contamination (Hole 1959).

The disease is classically introduced into a herd by the purchase of an infected animal, by diseased animals sharing pastures with healthy animals, and by drinking contaminated water from ponds or slow-moving streams. It has been documented that calves less than 30 days old are more susceptible to infection than older animals (Larsen 1975, Rankin 1959, 1961). Experimental work would suggest most cattle are infected before they are 4 months of age (Hagan 1938, Taylor 1953). Most infections therefore occur early in life with clinical disease occurring 2-5 years later, which emphasizes the prolonged incubation period (Doyle 1953).

The Johne's bacillus has been isolated from fetuses and uteri of infected dams (Doyle 1958, Kopecky 1967, Larsen 1974, Lawrence 1956, McQueen 1979), but the available evidence suggests intrauterine or congenital transmission occurs and may be an important cause of natural infection. Infection is reported to be more likely in offspring of infected dams but calves may become infected due to the increased risk of exposure to contaminated feces within infected herds. Merkel showed that 75% of infected animals in one herd, however, were from noninfected dams (Merkel et al 1975). The increased risk of infection in calves of infected vs. noninfected dams has not been documented but is the

rationale for culling of calves from infected dams as a disease control measure (Moyle 1975, Pearson 1955).

M. paratuberculosis has been cultured from the milk of confirmed Johne's cases even when not present in the feces of the same cow (Taylor et al 1981). In the same study *M. paratuberculosis* was cultured from the milk of 26 clinical cases. Additionally, isolates were made from 3 other cows deep in the udder tissue (2 cows) or from the supramammary Lnn. Neither naturally infected milk nor the feeding of pooled colostrum to calves has been studied as the source of infection for calves. However, obviously this is a potential source of infection for calves and should be considered in any control program.

Small numbers of *M. paratuberculosis* organisms have been recovered from semen and associated reproductive structures (Larsen 1981). Additionally, the organism can remain viable in the uterus for some time with minimal effects (Kopecky 1967, Merkal 1982).

Environmental studies have shown the organism to remain viable in the soil for 11 months, in infected feces for 246 days and in pond water for 163 days (Lovell 1944). Growth is inhibited by the presence of urine and by the ensiling process (Hole 1958). One Danish slurry system for manure handling reported the survival time ranged from 98 to 252 days. Lower temperatures, moisture and absence of exposure to sunlight enhance environmental survival of the organism (Jorgansen 1977, Larsen 1956). To date, however, no studies have identified the role of environmental contamination as a source of infection. The importance of wildlife transmission to cattle is unclear, but wildlife may have a similar rate of infection where they share common grazing areas (Riemann 1979). Infected deer may serve as a source of infection for livestock (Chiadini 1983).

Infection and Disease

Following initial exposure to *M. paratuberculosis* cattle may reject the agent or maintain the infection at such a level so that there is no interference with productivity nor immediate risk of transmission to other animals in the herd. These animals are infected but resistant. Such animals may respond to immunological tests such as lymphocyte transformation or to the intradermal skin test. By contrast in some animals the organism progressively multiply resulting in clinical signs. These are obviously diseased. Between these two extremes, the resistant infected animal and the advanced diseased animal, an intermediate type commonly occurs. This is the basis for designating paratuberculosis a spectral disease (Duncan 1978).

The clinical disease is characterized by weight loss, mild diarrhea, and over a period of weeks more severe diarrhea and greater loss of weight, terminating in emaciation and death. The appetite usually remains good until the animal becomes terminal. Some animals may have no or intermittent diarrhea for a period of time (weeks or months) with loss of body condition, others develop secondary

diseases, such as mastitis, and are culled from the herd for reasons unrelated to diarrhea.

Although several animals in a herd may be *infected* at one time, usually only one or two animals show any clinical signs of illness at any one time over a period of 4-8 months. Those infected adult animals often shed organisms in the manure which may infect other susceptible animals, especially youngstock (less than one year old).

Physical examination usually allows one to divide the disease into three types which are:

Type I—Inapparent carriers—the largest percentage of infected cattle are in this category. These animals do not have diarrhea but may be immunologically abnormal and prone to other infectious diseases such as mastitis and infertility. These animals may be a threat to other animals on the farm by virtue of fecal contamination. This form of the disease may be the most costly to the owner by infecting cattle unknowingly and decreased production in infected cattle. Many of these animals are negative on fecal culture.

Type II—clinically diseased with a typical watery pea-soup fluid feces but no blood or tenesmus. The diarrhea can and often is intermittent. The vital signs are normal. Emaciation and cachexia develop and milk production decreases. Appetite remains excellent but thirst is increased. Most of these animals are positive on fecal culture and on some serological tests.

Type III—advanced clinical disease. These animals are weak, emaciated, usually have a profuse diarrhea with obvious bottle jaw. At this stage they may not pass inspection for meat (human consumption). Animals can progress from Stage I to Stage III in 2-4 weeks and die or rarely never show diarrhea.

Clinicopathological Findings

The abnormalities reported are often characteristic of Johne's but certainly not diagnostic. Typically animals in advanced disease are hypoproteinemic with reduced total serum proteins of all types (Patterson 1967, 1968 and Rice 1969). Studies with protein markers suggested plasma proteins leak across the gut mucosa to the lumen of the bowel (protein-losing enteropathy). In-vitro studies of diseased intestinal mucosa also reported a decreased uptake of the amino acid-histidine (Patterson 1969). The decreased absorption of nutrients and loss of plasma proteins on occasion leads to a rapid catabolic state with increased muscle protein catabolism (Nielson 1966). The marked muscle loss may be associated with elevated plasma phosphorous levels (up to 12 mg/dl).

Animals in advanced stages of Johne's are often anemic with concurrent low values of calcium, sodium and potassium. Early stages of infection are not associated with any characteristic biochemical or enzymatic changes (Patterson 1965).

Resistance

Age—neonatal calves are highly susceptible. Cattle over

two years of age are not readily infected even when introduced into a contaminated environment (Rankin 1962). Thus, with each additional month of age the animal becomes more resistant, however, large infecting numbers of organisms will overcome the age resistance (Chandler 1961, Rankin 1958, 1961, 1962).

Breed—the prevalence is higher in some breeds than others and is higher in dairy than in beef cattle but all breeds of cattle, sheep and goats are susceptible. Goats show few clinical signs other than weight loss (emaciation). Diarrhea is rarely a sign of Johne's disease in sheep or goats.

Disinfectants—Orthophenyl phenate is one of the best. The trade name is "One Stroke Environ" by Vestal Laboratories. However, this disinfectant is less efficient in the presence of organic matter. Two to three percent cresol disinfectant with a detergent is also a good alternative to "One Stroke Environ."

Tests for Johne's Disease

Although the clinical signs of Johne's disease are often characteristic, i.e., weight loss, diarrhea with a normal appetite, other diseases may mimic Johne's especially parasitism and renal amyloidosis. Additionally, many Johne's **infected** cows may not develop diarrhea or weight loss but because of decreased immune responsiveness are predisposed to mastitis, infertility or other problems (Merkal 1975). Thus, a definitive diagnosis for Johne's disease must be made in the laboratory.

Fecal culture is the most specific test for Johne's disease. However, the required culture time of 3-4 months lessens the diagnostic utility. Most cattle positive on fecal culture are likely to develop clinical signs within a few months. Infected animals represent a hazard to in-contact animals especially young calves (the most susceptible age). Thus, once identified as fecal culture positive the animal should be slaughtered.

Fecal samples should be taken directly from the rectum using a fresh plastic sleeve for each animal. Lubricants for the plastic sleeve should not be used as this may interfere with the culture process. The fecal samples should be placed in one ounce plastic or metal specimen containers labeled with the animal's identification number. Samples should be shipped without freezing or refrigeration as soon as possible after collection to the laboratory. Specimens should be collected early in the week so they will not be held in the post office over the weekend.

Once the samples are received by the laboratory they should be processed as quickly as possible in a manner recommended by the Mycobacteriology Laboratory, National Animal Disease Center, Ames, Iowa (Whipple 1985). An egg yolk agar media (Herrold's) containing ferric mycobactin J (2 mg/liter) and sodium pyruvate (4.1 g/liter) is preferred. During the 16-week incubation period the tubes are examined with a dissecting microscope for colonies with morphology and growth rate consistent with *M.*

paratuberculosis. Those colonies that are acid fast, mycobactin dependent, and with morphology and slow growth rate are considered to be *M. paratuberculosis* (Whipple 1985). Occasionally (5-10%) individual samples or most of the samples from a herd will be overgrown with fungus; this has been associated with feeding moldy feed. If such feed is fed, it should not be fed 48 hours prior to collecting the samples.

Skin tests (intradermal) are of minimal value even though Johnin is used in preference to avian tuberculin (Gilmour 1976). Although this test may be required by some countries, it is of no value to identify positive fecal shedders (too many false positive and false negative tests) (Bendixen 1978).

Serological tests including complement fixation, ELISA, AGID, lymphocyte transformation, immunoelectrophoresis, have been used for the diagnosis of Johne's (Jorgensen 1979, Thoen 1977, DeLisle 1980). The AGID test, when positive, means the cow will likely be shedding organisms (Sherman 1984). Most other serologic tests have false positives as well as false negatives, thus making it difficult to cull cows with a positive test. The principle goal of several laboratories at this time is to develop antigens which are both sensitive to detect infected cows and highly specific with no false positive tests.

Tissue (intestinal) examined with a microscope is an accurate test but requires surgical intervention to obtain a biopsy of the intestine or lymph node or tissues can be taken when the animal is slaughtered. This test is sensitive and specific, but not practical on a herd basis. Rectal biopsy examination of clinical cases found a high correlation with clinical cases (Hoffsis 1983).

The Commonwealth of Pennsylvania, Department of Agriculture, has recognized Johne's disease as an important disease of livestock for many years and is a **reportable** disease in the state. The true prevalence of the disease is unknown as no comprehensive survey has been done in the state. The Pennsylvania State Diagnostic Laboratory at Summerdale is currently filled to capacity (200 cultures per week) with requests for Johne's cultures, which indicates the level of concern the producer and veterinarian has for Johne's disease. Since indemnity may be paid for Johne's positive cows by the BAI, the direct cost of Johne's disease to the state is substantial, i.e., greater than \$100,000/year. Johne's disease has become recognized as a major disease that is deserving of national attention.

Blood sample, fecal sample and tissues from the ileum, ileocecal colic lymph node and rectum were collected from about 1,400 adult Holstein dairy cows from a major slaughterhouse in northeastern Pennsylvania (Taylor's Packing Company, Wyalusing, PA). This plant processes more than 200,000 animals per year and accounts for the processing of more than 10% of the culled dairy cows in the United States. Several days (3-5) each month a research team visited the slaughterhouse to collect the specimens from randomly killed cows (approximately every 12th animal in the processing line for that day). Approximately

120 animals were sampled each month for a year.

The prevalence of Johne's disease in slaughtered cows was determined by a collective assessment of the culture results from the three specimens obtained from each cow—ileocecal lymph node, ileocecal valve and feces.

Only adult female Holsteins were evaluated for Johne's and the following data was obtained from each animal sampled at the slaughterhouse:

1. Live weight
2. State eartag number(s)
3. Backtag identification number to permit traceback to the auction the animal was purchased.

Using the Pennsylvania state eartag number(s), the Bureau of Animal Industry (BAI) helped to locate the herd of origin in the state. This step provided the following information:

1. Owner's name, address, phone number, and BAI herd number. The herd owner was then mailed a detailed questionnaire.

Results

The overall prevalence was 7.2% with 88 culture positive at any one site of the 1,224 cows sampled over the year long study. The prevalence on a monthly basis was similar for all months except June when nearly 25% of the cows both from Pennsylvania and from the other states were positive. The reason for the seasonal occurrence is unknown.

The sale tag permitted the determination of the state of origin of each cow. The prevalence of Johne's positive (based on culture) cows from each state is shown in the following table:

TABLE 1.

State of Origin	Cows Sampled	No. Positive	% Positive
Connecticut	24	1	4.2%
Delaware	4	0	0.0%
Indiana	3	0	0.0%
Massachusetts	25	1	4.0%
Maryland	50	8	14.0%
Maine	2	0	0.0%
Minnesota	1	0	0.0%
Missouri	1	0	0.0%
North Carolina	21	1	5.0%
New Hampshire	7	0	0.0%
New Jersey	23	1	4.0%
New York	311	15	5.0%
Ohio	91	17	19.0%
Pennsylvania	502	36	7.2%
Vermont	52	1	2.0%
Tennessee	2	1	50.0%
Virginia	64	4	6.0%
Unknown	8	0	0.0%
West Virginia	21	1	5.0%
Ontario	4	1	25.0%
Overall	1,224	88	7.2%

The cow's origin on the farm was identical for both positive and negative groups of animals, i.e., 77% were home raised while 23% were purchased. Of those purchased animals, about 17%-19% were from auction; 17%-22% came from dealers while 67%-59% were obtained from other farms for positive and negative cows respectively. The age at purchase was not different for positive and negative animals where 60% and 69% were brought to the farm after calving.

It is not easy to assess management practices with a questionnaire but one attempt to assess this was made by the determination of participation in the DHIA program. One might suspect those herds on DHIA might have better management. The differences were not significant between positive and negative cows, 40 and 51% respectively.

In as much as many investigators believe the first few weeks of a calf's life is thought to be critical, questions about nursing and contact time of the calf with the cow was assessed. Nearly 70% of both groups of cows were allowed to nurse their calves. However, only 40% of the positive cows were separated from their calves before 12 hours while 52% of the negative calves were separated at 12 hours. During the next six weeks about 55 of the calves from both groups of calves were housed in the same barn as the dam. A similar proportion continued to be housed in the same barn from weaning to 6 months of age.

Economic Losses Attributed to Paratuberculosis

The economic losses associated with Johne's disease have been attributed to a variety of factors including: premature culling; poor feed conversion, decreased milk production; increased calving interval and increased predisposition to other diseases. Abbas, (1983) found infected cows gave 1,838 lbs less milk on a 3-5 mature equivalent basis and had a longer calving interval (1.7 months) than paratuberculosis negative cattle. Mastitis as a reason for culling cattle in 3.6% of non-infected cattle compared to 22.6% of cattle with inapparent paratuberculosis (Merkal 1975). Infertility was a greater problem in infected cows compared to non-infected cows in the same study.

The estimated cost of paratuberculosis to Wisconsin farmers was \$52,396,012/year (Arnoldi 1983). The total losses were estimated to exceed \$1.5 billion annually for the dairy industry in the United States (Merkal 1984). Buergelt (1978) reported clinically affected cows gave 2,736 lbs less milk for the 305 day lactation than non-infected cows in the same herd. Our slaughterhouse survey gave similar results, 3,400 lbs less milk for the last lactation compared to non-infected cows. A conservative annual loss for Pennsylvania is \$5.8 million/year for the 700,000 dairy cows in the state (Whitlock 1985). Whatever calculations one uses, the losses attributable to Johne's disease are staggering.

Prevention

Paratuberculosis may be minimized by recommendation of the following procedures:

- a. Remove newborn calves from their dams immediately after birth and raise them in separate quarters. This is the most important recommendation (Julian 1975).
- b. Do not allow the calf to nurse from an infected cow; while nursing the calf may ingest *M. paratuberculosis* bacilli from fecal contamination on the udder or teats (Pearson 1967).
- c. Feed pasteurized colostrum from uninfected cows immediately after birth. Make sure the udder is clean prior to taking the colostrum. Pasteurization 145°F for 30 minutes or 165°F for 15 seconds. The Dairy Goat Journal recommends heating colostrum to 131°F for one hour. Sears sells a commercial pasteurizer which reportedly will do an effective job on colostrum (it is a messy job to say the least).
- d. Separate unthrifty cattle from the herd and handle these cattle last. Return them to the herd only when known negative for Johne's.
- e. Protect young cattle from contaminated feed and water used for feeding adult animals. This can be accomplished by separation of different age groups of livestock.
- f. Do not mix replacement animals with the adult herd until they are at least two years old.
- g. Keep feeding areas (feed bunks, etc.) well above ground to minimize contamination with feces.
- h. Do not spread manure on permanent pastures used for grazing cattle, especially youngstock.
- i. Contaminated areas, lots, pastures should be plowed by turning over at least 6 inches of soil.
- j. Rotate pastures to prevent adult animals from ingesting the infectious organism.
- k. Protect young cattle from all waste and water drainage that may come from areas occupied by adults.
- l. Fence off or fill in any stagnant water source. Allow cattle to drink from only tanks or free flowing streams.
- m. Slaughter any animal with recurrent diarrhea.
- n. Culture all adult animals at six months intervals if your herd has Johne's suspects. Any positive animal should be sent to slaughter immediately.
- o. Purchase animals only from clean herds without a history of Johne's disease.
- p. A vaccine is available but should be considered only when all other measures are not effective or possible to control Johne's disease.
- q. Sale of known positive Johne's cattle may cause the owner to be subject to civil liability. If cattle are sold from herds with Johne's disease, the owner has a legal obligation to inform the buyer these cattle have been **exposed** to Johne's disease. If the seller does not so inform the buyer, he is open for civil liability.

Vaccine

A vaccine is available for restricted use according to state-

federal guidelines. The vaccine is restricted for use by an accredited veterinarian usually under supervision by the state veterinarian. Not all states permit the use of the vaccine. Approval for the use of *M. paratuberculosis* bacterin, USDA License No. 195A, Fromm Laboratories, on a specific farm is dependent upon the following conditions:

1. Review of the prevalence of Johne's disease in the herd must indicate uncontrolled spread of the disease and the attending herd veterinarian must recommend the use of the vaccine.
2. A Memorandum of Understanding (MOU) outlining obligations and responsibilities of the Herd Owner, Herd Veterinarian and the state veterinarian must be endorsed by the three major participants.
3. Only accredited veterinarians instructed by the state veterinarian's office will be authorized to possess and administer the bacterin.

Veterinarians interested in the Johne's Disease Calfhood Vaccination Program should contact their state veterinarian.

Efficacy:

Preliminary data indicates the vaccine is effective in reducing the incidence and delaying the onset of clinical signs (Larsen 1978). The vaccine does not totally eliminate infection (Doyle 1960, Hurley 1983, Larsen 1969, 1973, 1974, Stuart 1965). Some vaccinated animals may be normal clinically but have infected intestines, shed organisms, and have positive fecal cultures. Animals must be less than 35 days old when vaccinated. The owner must agree to vaccinate all animals born on the farm. Animals over 35 days of age cannot be vaccinated. Since 1969 about 20,000 calves have been vaccinated in Wisconsin. The infection rate in these herds has dropped from 11.7 fecal shedders per 100 cows to 0.8 fecal shedders/100 cows per year (Arnoldi).

Adverse Reactions associated with use of the vaccine occur commonly and include:

- a. A lump (granuloma) forms at the site of the injection (dewlap) of the vaccine which may vary from one inch in diameter to several inches in diameter. Occasionally, one will break open and drain for a time. The drainage material is pus and **not** infective *M. paratuberculosis* organisms as the vaccine is killed.
- b. Some Johne's vaccinates react positively to the TB test. Such reactions can be differentiated from TB infected cattle by the comparative cervical TB test. Herds must have negative TB status before Johne's vaccination is instituted.
- c. The vaccinate may be positive on subsequent Johne's serologic tests, such as the complement fixation, AGID or ELISA tests.
- d. Occasionally veterinarians have injected their finger which later required amputation. This has lead some veterinarians to refuse to vaccinate cattle because of fear of

self-injection.

e. Vaccinated animals must be tattooed with a special identification 1, 2, 3, or 4 (for the quarter of the year), J (for Johne's) and two digits for the year.

f. Animals from vaccinated herds may require the health certificate to say they originate from a Johne's infected herd.

Recently an organism resembling *Mycobacterium paratuberculosis* has been isolated from several patients with Crohn's disease (Chiodini 1984, Thayer 1984). Since that original publication a spheroplast (cell wall deficient organism) or *Mycobacterium paratuberculosis*-like organism has been isolated from additional patients in several different laboratories worldwide (Australia, France and USA). Patients with Crohn's disease had a significantly higher antibody titer to *M. paratuberculosis* than did control patients or patients with ulcerative colitis (Thayer 1984). Research is actively pursuing a possible link between Crohn's disease and *M. paratuberculosis*.

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