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DEVELOPMENTS IN VETERINARY SCIENCE

A SHORT REVIEW AND SOME OBSERVATIONS ON JOHNE'S DISEASE WITH RECOMMENDATIONS FOR CONTROL

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INTRODUCTION

JOHNE'S DISEASE has been described as one of the most serious diseases affecting the world cattle industry at the present time (123); however, it is not the serious and widespread problem in Canada that it is in Great Britain and some countries in Europe. In England and Wales, it is of great economic importance (14) and the most important disease of cattle in certain areas (16, 20). A 1959 report from Britain based on culture of mesenteric lymph nodes from slaughter cattle carried out by different investigators between 1947 and 1959 indicated that 11% of all cattle were infected (122). Other surveys revealed that 20–30% of cattle sent to knackeries or to casualty slaughter had Johne's disease (79, 95, 96). The disease is considered to be enzootic in many countries in Europe including Holland (39, 41), Belgium (13), France (22), Denmark (44) and Russia (81).

Johne's disease is widely distributed in the United States and the reported incidence has increased greatly over the past 22 years, probably due both to an actual increase in the disease and to improved diagnostic methods and reporting (50).

The actual herd and individual incidence of Johne's disease in Canada is unknown but it does exist across the country at a low level (23). The pathology records at the Ontario Veterinary College show that 52 cases of Johne's disease have been confirmed in the past 24 years. Most of these represent individual infected herds since, once the diagnosis has been established, further cases are rarely submitted. As infected herds tend to remain infected and movement of cattle has increased considerably in recent years, the incidence of the disease is undoubtedly increasing in Canada. In 1958, Konst (47) citing a federal government report (35) stated that the yearly discovery of a considerable number of new foci of infection indicates that a gradual and unhalted dissemination of the disease is taking place. The disease is not reportable in Canada but there is a voluntary tested herd program (78). In 1936, the first year of the program, 25 herds were enrolled and in 1973, 65 herds were enrolled. The program involves compensated slaughter of clinically ill animals as well as those positive to the intradermal johnin test together with the cleaning and disinfection of premises. There are no quarantine restrictions, and cattle which are negative on test can be moved in and out of the herd or sold. Some enrolled herds have eventually become negative (23).

Although Johne's disease may not be a major problem in the Canadian cattle industry, it does cause serious economic loss in individual infected herds. Therefore, it is of primary importance to prevent the spread of this disease from infected to non-infected herds.

Epidemiology

Johne's disease is caused by infection with *Mycobacterium paratuberculosis* (*M. paratuberculosis*). Although a variety of species of animals can be infected experimentally and under unusual circumstances, the disease occurs naturally only in domestic and wild ruminants. In Europe and North America, it is of economic importance only in cattle, sheep, and

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goats (9). There is one report of natural disease in a pygmy ass in a zoo (120) and one of suspected Johne's disease in pigs (112.) There are reports of lesions but not clinical disease in naturally infected swine (110) and in a horse (97). Experimental infection has produced lesions in chickens (64) and both lesions and clinical disease in swine (43, 63), horses (65) and a variety of laboratory animals (9).

The organism has a predilection for the intestinal tract and, according to Gilmour (24, 25), the intestine is the site of primary infection and the main site of bacterial proliferation and action. In ruminants, clinical disease results only after intestinal lesions develop. Gilmour states that infection becomes established in the intestine of all calves that ingest a sufficient number of organisms. Payne (92) reports similar results in adults given the organism for the first time. Lesions are produced within one to two months in the intestinal mucosa and lymph nodes of calves, and large numbers of organisms may then be shed for a varying length of time. Following multiple oral dosing, Payne (91) found that lesions and excretion of organisms reached a peak at three months in calves and two months in cows. Following single dosing, Larsen (67) found that organisms were shed for only about 30 days starting approximately 30 days following dosing. Clinical disease did not occur during the time animals were kept, but this was shorter than the normal incubation period. Animals were not held to see if they remained as carriers or to see if they would have become shedders again.

Following the development of lesions, the organism may be carried from the intestinal mucosa and perhaps from the tonsil, retropharyngeal lymph nodes, and nodes along the digestive tract to other parts of the body in macrophages (25, 46, 91). The organism has been recovered from a variety of organs and body secretions from both clinical and nonclinical cases (99). It has been known for many years that congenital infection is possible (36) and more recently uterine and fetal infections have been found to occur fairly frequently even in cattle not showing clinical disease (48, 68, 93). Johne's disease has also been reported as a cause of abortion (80). The mammary gland may become infected but not as commonly as the uterus (15, 113). The organism has been found in seminal fluid, sperm, and testes (62, 68, 119).

Under natural conditions, infection is usually oral following ingestion of the organism in water or feed contaminated with infected feces, or by nursing from an udder or bucket contaminated with infected feces. The disease is most likely to become serious under conditions of heavy cattle population where calves are in close contact with the adult herd.

M. paratuberculosis has been described as being of low pathogenicity for the various species in its normal host range (9). Low pathogenicity may be the reason for the long incubation period and for the age resistance that has been noted in natural and induced disease. According to Hagen (34), clinical signs are rare in calves which become infected after four months of age. In fact, extremely heavy exposure did not produce disease in animals over two years of age. Rankin (99, 100) however, reported clinical disease in two calves naturally exposed at six months of age and in one cow naturaly exposed at four years of age. It must be remembered that clinical disease is not common under natural conditions, being observed in only 3–5% of the animals in an infected herd (122) in spite of the high proportion of apparently normal adult cattle harbouring or shedding the organism. In infected herds, carrier animals are up to 20 times as frequent as clinical cases (122) so it is possible that a very high percentage of cattle in an infected herd are carriers. Rankin (98, 99) recovered the organism from the mesenteric lymph nodes of all of five intravenously and nine naturally infected cows four years after exposure. Of the carriers, some will be nonshedders, some light shedders (organism culturable from intestinal mucosa at slaughter but not from feces because there are less than 100 organisms per gram of feces), and some heavy shedders (organisms culturable from the feces) (66). Heavy shedders usually become clinical cases within one to two years. Some light shedders also become clinical. The age at which diarrhea develops is variable (Table I) depending perhaps on the various factors affecting susceptibility to the organism, and may be less than one year (36).

Many factors influence the development of clinical disease following infection (9). Age at the time of first infection may be the most important (8, 16, 34, 98, 100). Size of the first and subsequent doses of the organism may be significant (9, 98). With this disease, perhaps more than most, factors affecting individual susceptibility such as the stress of poor nutrition, concurrent infection (parasitism), heavy milk production, or shipping may be important determinants of clinical disease. Hole (37) feels that familial susceptibility is a significant factor and that certain families in a herd are more apt to develop clinical disease. Breed susceptibility has also been discussed.

TABLE I

AGE DISTRIBUTION OF CASES DIAGNOSED AS JOHNE'S DISEASE AT THE ONTARIO VETERINARY COLLEGE 1950–1974

Age at time of submission	Number of cases
1½	3
2	5
3	5
4	7
5	6
6	4
7	4
8	3
9	3
10	1
Age not stated	11
Total	52

TABLE II

BREED DISTRIBUTION OF CASES DIAGNOSED AS JOHNE'S DISEASE AT THE ONTARIO VETERINARY COLLEGE 1950–1974

Breed	Number of cases
Shorthorn	18
Jersey	8
Hereford	5
Angus	5
Holstein	3
Guernsey	1
Breed not stated	12
Total	52

In a survey in England, the incidence in Channel Island breeds and Shorthorns was higher than in other breeds (16, 122). A survey in Denmark (44) revealed that the infected herds were predominantly Jersey. In Minnesota, 36% of the infected herds were of the Shorthorn breed although this breed made up only a small percentage of the total cattle population (114). In the cases submitted to the Ontario Veterinary College, the Shorthorn breed had the highest incidence (Table II).

It has been suggested that the apparent family and breed susceptibility to clinical disease may be due to increased exposure rather than increased susceptibility (8). Chandler (9) has reported that different breeds of mice vary in susceptibility to infection with the Johne's bacillus. Different strains of the organisms apparently do not vary in pathogenicity for cattle (36). Route of infection is significant in experimental animals and sheep, with the parenteral route superior to oral dosing in the production of lesions (9, 46). The significance of congenital or possible inhalation infection in cattle has not been examined. If age is a factor in the subsequent development of clinical disease, congenital infections may be quite significant. Soil acidity is reported to predispose to clinical disease in cattle (42) as is phosphorous deficiency (22).

Clinical Signs and Pathogenesis

The typical clinical picture includes progressive unthriftiness, weight loss, decreased milk production, with the development of continuous or intermittent diarrhea. Fecal consistency varies from soft to watery with the latter animals tending to dehydrate and die quickly. As the syndrome progresses, muscle wasting, weakness, dependent edema, and inappetance are often observed. Fever and anemia are not always present. Although the onset of clinical disease may coincide with pregnancy or heavy milk production, these stresses probably just hasten the onset of clinical disease that would have been inevitable in that animal. Two cases were confirmed as Johne's disease at the Ontario Veterinary College during the past year. One of these was a five-year-old Angus bull which had been losing flesh for a year and had had diarrhea for several months when submitted. The bull was still strong and in fair condition, and might have survived another year or two. However, he was euthanized following confirmation of Johne's disease by surgical biopsy of lower ileum and ileocecal lymph node. This bull had been purchased as a yearling and was used as a herd sire for three years before developing signs. There was no previous history of Johne's disease on the farm.

The second case was a four-year-old Shorthorn cow that had been unthrifty since calving three months earlier. She became rapidly dehydrated upon developing diarrhea and died after only four weeks of obvious illness. Although Johne's disease had not been confirmed previously on this farm, the owner reported that he lost one or two cows with diarrhea each year and this was the third case this year.

These two reports represent the chronic and acute forms of the disease. They also represent the farm where infection may have been present but undiagnosed for many years, and the situation of a recently introduced animal which probably infected a previously clean herd.

The pathogenesis of Johne's disease is not yet definitely established although there have been several suggestions as to how the organism produces diarrhea, the primary clinical sign. Merchant (69) states that packets of organisms appear in the mucosa and act as

foreign bodies producing a specific inflammatory reaction. Patterson (84, 85, 90) suggests that the massive cellular infiltrate in the mucosa and submucosa results in increased gut motility, decreased transit time, decreased absorption and has shown an increased loss of protein from the intestine. More recently, Merkal (72) has demonstrated that diarrhea may result from antigen-antibody reactions in the infected intestinal tissue with release of histamine in the tissue. Parenteral use of antihistamines or histidine decarboxylase inhibitor usually were each followed by reduction or cessation of diarrhea in clinically ill cattle. Bellamy (7) has suggested that the intestine can withstand antigen-antibody reactions under normal circumstances and when lymph channels are not occluded because the vascular system is normally adequate to remove released neutrophil lysozomal enzymes and to prevent edema. In addition many of the neutrophils emigrate into the lumen rather than breaking down in the mucosa. If this is so, the cellular infiltrate and lymphangiectasia in Johne's disease may impair this mechanism making the intestine hypersensitive to immune-mediated reactions. Merkal (75) also suggests that cell-mediated immunity may cause the febrile response, emaciation, and anemia which occur in some cases of Johne's disease and he has shown that johnin hypersensitivity can be passively transferred to produce fever and diarrhea following administration of johnin in calves passively sensitized with whole blood or with blood cells from an artificially hypersensitized bull.

Lesions

The gross and microscopic lesions have been described repeatedly, and excellent accounts are available (45). However, it should be noted that gross lesions are frequently not present so that microscopic or cultural confirmation is necessary in all cases. Recently there has been a report of the isolation of *M. paratuberculosis* from a calcified lymph node in a cow (107). It had been felt that this type of lesion did not occur in cattle. There have been many investigations into changes in body fluids and tissues in clinical Johne's disease in an attempt to find tests to assist clinical diagnosis. Although abnormalities are present in cellular components of blood (3, 105), in serum protein (10, 86, 106), enzymes (82, 83, 89), electrolytes (19, 22, 116), and in muscle (87), liver (88) and intestine (85), diagnostic changes are present only in intestine and intestinal lymph nodes and these are morphological rather than chemical differences.

Diagnosis and Control

The diagnosis of clinical Johne's disease in a herd of unknown status is difficult. The differentiation of carrier animals in a known, positive herd is even more difficult (72). The unreliability of the various diagnostic tests has been given as the reason for not making the disease notifiable (94). Since, with our present knowledge, identification of carrier animals is almost impossible, elimination of the disease from infected herds should not be the primary goal, although control of clinical disease in infected herds is important. *Prevention of spread of Johne's disease from infected to non-infected herds should be the most important consideration in a control program.* If Johne's disease has not previously been diagnosed in a herd, positive confirmation of suspected cases is essential. The delayed hypersensitivity (johnin) and serologic tests are not adequate for this purpose (37, 71) although they would be useful on a survey basis to select herds that require further investigation. At the present time, only histopathologic or bacteriologic methods as described below would be considered diagnostic in a herd of unknown status. Negative results would not necessarily mean that the herd did not have Johne's disease.

Diagnosis in a herd of unknown status

1. *Histopathological diagnosis* – Typical lesions (macrophages, giant cells, plasma cells, and lymphocytes in varying numbers, along with acid-fast organisms in macrophages) in the lamina propria of the intestine and in intestinal lymph nodes removed by surgical biopsy or at slaughter or necropsy.

If only one part of the intestine is to be examined, the terminal ileum should be selected. The biopsy method of diagnosis has been used at the Ontario Veterinary College, at the Western College of Veterinary Medicine, and by practitioners to confirm suspected cases of Johne's disease. Surgical biopsy of the ileocecal lymph node has been recommended by Rines (108) as a method of making a positive diagnosis of Johne's disease.

2. *Bacteriological diagnosis* – Culture of *M. paratuberculosis* from feces of suspected clinical cases or shedders, or from intestinal mucosa or lymph nodes at slaughter or necropsy.

An improved technique for fecal or intestinal culture has been described recently (70, 73). The method is not simple, and cultures must be incubated for three months; however, it has been suggested that fecal cultures for *M. paratuberculosis* can be done in any laboratory

that prepares its own media (77). Some laboratories that have tried fecal culture for *M. paratuberculosis* have had problems with media preparation; however, it is the only test in addition to histology that does not give false positives so it could be used to confirm the diagnosis in herds of unknown status. According to Merkal (76) it is also the most suitable diagnostic test for detecting infection in apparently healthy cattle in known infected herds.

Diagnosis in a known positive herd

A variety of tests have been developed to identify infected animals. None of these are specific enough to confirm Johne's disease, but positive results would indicate that one of the more specific tests should be carried out. These tests also have some value in selecting animals for disposal in a known positive herd (37).

1. *Delayed hypersensitivity reactions*

(*a*) The skin test using intradermal johnin. An antigen, such as johnin PPD (47), prepared from the organism is injected intradermally. In a sensitized animal, a localized cellular response occurs with thickening at the injection site (76). The test is usually read at 48 hours. This is said to be the best test to identify positive animals before clinical disease develops (37) and is widely used, however it is of doubtful diagnostic value (55).

(*b*) Systemic tests using intravenous johnin (3, 59).

(i) Temperature response.
Intravenous injection of johnin produces a temperature increase in sensitized animals. A rise of 1.5 degrees F six hours following injection is significant. This test is said to be the most valuable for differentiating clinical Johne's disease from other causes of chronic diarrhea (66).

(ii) White blood cell response (49).
Intravenous injection of johnin produces a stress or cortisone type response in sensitized animals with an increase in neutrophils and a decrease in lymphocytes. A positive result would be indicated when the ratio of neutrophils to lymphocytes six hours after injection is more than twice the ratio of neutrophils to lymphocytes before injection (76).

(*c*) Leukocyte migration inhibition (1, 5).
The addition of antigen (johnin) causes sensitized lymphocytes to release a substance that inhibits macrophage migration. The test is done on white blood cells taken from animals suspected of having Johne's disease. Animals which have antibody on their lymphocytes (sensitized lymphocytes) would be positive on this test.

2. *Serological tests for evidence of circulating antibody in serum*

(*a*) The complement-fixation (CF) test (102, 103, 104). Complement-fixing antibody may not develop until the infection is well established, and the circulating level of antibody usually diminishes after the development of clinical disease (8, 56) (perhaps the antibody is combining with antigen in the intestine). This is the only serological test that is widely used for diagnostic purposes and has been considered to be the best test available to identify suspected Johne's disease cases and carriers (37, 109) although it is not really satisfactory for this purpose (56, 76, 101).

(*b*) The passive – hemagglutination test. This test has been examined by Larsen (57) who states that it is as sensitive, but less specific than the CF test.

(*c*) The gel-diffusion precipitin test. This test is quite sensitive and has good specificity for sheep and goats. It is not satisfactory for cattle (76).

(*d*) The fluorescent antibody test. This test was developed by Gilmour (27, 29, 30, 33) to detect antibody in serum. Experimentally, it compares favourably with the CF test.

3. *Microscopic examination*

(*a*) The examination of feces or rectal scrapings for acid-fast organisms has been widely used and recommended by several authorities, particularly to pick out shedders in a known positive herd (16, 37, 109). However, Merkal (71) states that it is impossible to distinguish the Johne's bacillus from other acid-fast organisms which are frequent in feces. The acid-fast hay bacillus is usually larger than the Johne's organism and an experienced person might distinguish it; however, it is not possible to distinguish *M. paratuberculosis* from the myobacteria that are present in waterers and elsewhere in the environment (6). The finding of clumps of organisms in feces would be significant

in a known positive herd. Rectal scrapings are of doubtful value as they are positive only in longstanding clinical cases (16).

(*b*) The examination of smears from scraping of the mucosa of the terminal ileum taken at necropsy or slaughter is a satisfactory diagnostic technique (54, 76). The finding of clumps of acid-fast organisms or organisms in macrophages would be considered diagnostic in known positive herds or for routine survey purposes.

A recent comparison of tests for Johne's disease in 370 cattle from a known infected herd produced the following results (71). Compared to fecal culture, the CF test resulted in almost as many false positives (73%) as positives (89%) at 1:8 dilution and gave positive results on less than half of the fecal-culture positive cattle at a 1:32 dilution. The intradermal johnin test was even less sensitive and only slightly more specific. Microscopic examination of feces revealed organisms resembling *M. paratuberculosis* in 91% of culturally-positive cows and in 83% of necropsy-positive cases; however, 76% of culturally and necropsy-negative cows also had organisms in fecal smears which resembled *M. paratuberculosis*. The results of fecal culture corresponded quite well to examination of smears from scrapings and culture from intestinal mucosa of animals disposed of for Johne's disease or other reasons. In the case of the serological and allergic tests, probably some of the animals that were recorded as false positives did in fact have antibody to *M. paratuberculosis* since they were in an infected herd. However, these tests would not appear to be satisfactory for identifying shedders, which are the potentially dangerous animals in a herd. The tests would be of value for distinguishing potentially positive or negative herds (59) but, even in a negative herd, false positive reactions can occur.

Differential diagnosis has been described in a variety of articles and texts. Johne's disease could be confused with a long list of diseases that cause weight loss or diarrhea; however, those listed by Jubb and Kennedy (45) might present the most difficulty in differentiation. It is important to consider the possibility of Johne's disease in cases of unexplained chronic diarrhea in herds where the Johne's disease status is not known and to surgically biopsy or necropsy suspicious cases to confirm the diagnosis. In an infected herd, Johne's disease will automatically be suspected in poor-doing or diarrheic animals.

As with other diseases, the control of Johne's disease may be considered under four headings:

1. *Isolation and Sanitation*

(*a*) Negative herds can be kept negative by avoiding introduction of or contact with shedder animals. Community pastures or contaminated watercourses may be a source of infection.

(*b*) Management is effective in lowering the number of clinical cases in a positive herd (16, 36, 60, 111). It has been known for many years that good sanitary practices and certain management procedures (such as separating calves from the adult herd at birth and removal of heavy shedders) will lower the incidence of clinical disease. The Johne's tested herd program would be of value for this purpose. If possible, a source of drinking water should be provided that cannot become contaminated with feces. Since it is not likely practical to separate calves in a commercial beef herd, early removal of shedders and clinical cases and all their offspring would be of some value to lower the level of organisms in the environment. Beef herds, particularly those on range or large pasture areas, should not be brought together or into one place for calving. If possible, young calves should be in close contact only with their dams to prevent dissemination of infection from shedders to a large number of calves. The incidence of carriers and clinical cases in a herd depends largely on sanitary precautions.

2. *Avoid stress and predisposing causes*

A variety of factors may mean the difference between carrier animals and clinical cases in an infected herd. Care should be taken to avoid parasitism and to provide an adequate diet. The application of lime to acid soils may be of value as may the use of phosphate fertilizers (11, 12).

3. *Vaccination*

Although vaccination has not been effective in all cases (38) it has been shown to prevent most clinical disease in infected herds and to lower the level of organisms shed (17, 18, 40, 41, 115, 118). Vaccination is said to inhibit the intracellular multiplication of the organism (24, 66). Where vaccination is practised, one dose at one week of age is recommended. Vaccination against Johne's disease may sensitize animals to the tuberculin test; however, the vaccine reaction could be differentiated in most cases by newer test methods (2, 61, 117, 121).

4. *Treatment*

A variety of drugs have been tried to control clinical Johne's disease (21). Such things as streptomycin (51), iso-nicotinic acid hydrazole (53) and viomycin, 4–4′ diamino diphenyl sulfone (52) have been used experimentally without significant effect although temporary improvement may occur. Gilmour (26, 28, 31, 32) has studied riminophenazine B663 (G.30320) and found it effective in reducing infection in mice and sheep but not in calves. Using the same product, Merkal (74) was able to control but not cure clinical disease in cows. Phosporus has been recommended for the treatment of clinical disease even though affected animals usually have elevated serum phosphorus (19). Corticotrophin and anabolic steroids do not affect the course of clinical disease (4). At the present time, no specific medication is recommended for Johne's disease.

Discussion and Recommendations

Research into Johne's disease in Great Britain, Europe, and North America has increased our knowledge of the pathogenesis of the disease. There has been an intensive search for improved diagnostic tests that would distinguish animals shedding *M. paratuberculosis* in their feces from non-shedders or, more particularly, shedders from those which will never become shedders. The delayed hypersensitivity and serological tests will not adequately separate shedders and potential shedders from non-shedders. Even fecal culture will only pick out relatively heavy shedders (over 100 organisms per gram of feces) and has no value for selecting potential shedders. None of the tests are satisfactory to allow individual cattle to be certified as Johne's disease free (58, 76) although on a herd basis (if the herd were of sufficient size), negative intradermal johnin and CF test results should be suitable evidence to permit certification of the herd or of individuals in the herd. The testing program suggested to the United States Animal Health Association in 1970 by Merkal (76) for a "Paratuberculosis Qualified Herd" plan would appear to be too elaborate to be practical for routine purposes, but a similar program would be necessary before a known positive herd could be certified as negative.

A free market should not give an individual the right to sell animals that can spread a disease for which there is no adequate control and which may cause serious economic loss to the buyer. No one can guarantee that any animal from a Johne's disease infected herd will not spread the disease when introduced into a non-infected herd.

Considering the status of our present knowledge on Johne's disease and our lack of adequate tests to identify animals which are potential shedders of the infection, the following recommendations would seem appropriate:

1. Make Johne's disease a named disease so that when infected herds are identified they would be quarantined. While under quarantine, cattle could be moved only to slaughter.

   The identification of positive herds would not necessarily mean testing all herds in Canada, but the intradermal johnin test, plus the CF test when positive johnin tests occur, should be done on herds when Johne's disease is suspected. Perhaps buyers should have the right to request a negative herd test when purchasing breeding stock. Herds would not be classed as positive until histological or bacteriological confirmation of Johne's disease was completed. There are control programs involving quarantine regulations in some States and countries at the present time (114).
2. Continue a control program similar to the present Johne's tested herd policy for those positive herds in which the owners wish to try to reduce the level of clinical disease or eradicate the infection. Because of the difficulty of eradicating Johne's disease, perhaps the owner should also have the option of doing nothing.
3. Permit vaccination in positive herds. Since all animals will be going to slaughter, vaccination should be permitted on these premises. Vaccination would lower the level of clinical disease, particularly if used in conjunction with good management practices (24). Vaccination would have the disadvantage of making eradication a long-term project since it would have to be continued until the herd was culturally negative. The herd could not be certified free until the last vaccinated animals had gone to market and was johnin and CF negative.

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