

The Epidemiology of Johne's Disease

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Introduction

What does the epidemiologist concern himself with when studying an outbreak of Johne's disease? When the disease is first diagnosed in a herd many facts must be determined. First, how many animals are infected? This is difficult to determine as will be seen in the section on diagnosis. Second, where did the disease come from and what caused the breakdown? Since the natural incubation period has been determined (Hole, 1958), the investigator cannot look at past records of the herd and determine the source of the disease. All the records and facts about the traffic in and out of the herd must be studied as well as the origin of the herd if they are not native cattle. It must be determined whether there are any other natural hosts in contact with the animals or their environment. Third, the epidemiologist must determine what steps should be taken to prevent any further outbreaks of the disease and to control the spread to other animals and premises.

Agent *Mycobacterium paratuberculosis* (*johnei*) is a small acid-fast rod measuring about $.5\mu$ by 1.0μ . In feces and tissue they usually appear in clumps or small groups. For growth in culture, the organism requires a growth factor that can be supplied by killed acid-fast organisms; usually killed *Myco. pheli* is used in Dorset's egg

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medium. Immunologically the agent is related to *Myco. avium* and antigens of the two organisms, paratuberculosis and avium, are of equal reactivity when administered intradermally (Green, 1946).

Some facts pertinent to the epidemiology are the survival time and sensitivity to disinfectants. Larsen *et al.* (1956) found that the organism would live for 17 months in saline and neutral tap water, 14 months in tap water of pH 5.0 and pH 8.5, and in 1% gelatin in tap water. The organism could not live in bovine urine. The bacillus remained viable when maintained in a dry state for 47 months. The organism could stand a variation of temperature from -14°C to 38°C . In sunlight, the organism lived for 65 hours but not for 100 hours. Medium containing 20% feces or urine would inhibit growth of the organism. Vardaman (1954) found sodium orthophenylphenate would kill the organism in 15 minutes in a 1:200 dilution. This appears to be the best disinfectant.

Diagnosis

In the control of any disease, the first step is to diagnose the disease accurately. This is particularly true in Johne's disease in which the main signs are diarrhea, decrease in milk production, and loss of condition. Many living, chemical and physical agents can produce the same syndrome. Therefore, it is necessary to have methods

by which the disease can be diagnosed in all animals of all ages. The methods of diagnosis and their efficacy will be discussed in their normal order of use — namely, skin test, mucosal scraping and fecal smear, serological test and finally demonstration of the organism.

Skin Test The skin test using johnin is the main diagnostic test employed in the field by the veterinarian. This test is applied similarly to the tuberculin test; and the johnin is prepared similarly to tuberculin. This is described by Larsen *et al.* (1955).

However, the efficacy of the test has been challenged, Rankin (1958) and Hole (1958, 1959). They believe that the test is of little value since the reaction frequently does not appear during the last stages of the disease. Hole (1958) cited figures to show that many reactors did not harbor the organism. Van der Schaaf and Zantinga (1955) reported that only 94 of 163 reactor cows were infected whereas 24 of 30 non-reactors were infected. However, Van der Schaaf did not say whether the diagnosis was based on gross lesions or on demonstrating the organism in a tissue section or smear. Diagnosis based on gross lesions alone is not adequate.

Larsen and Vardaman studied a herd of 190 cows, and examined 34 animals at post mortem. There were 19 reactors and 15 non-reactors. Small acid-fast organisms were demonstrated in 10 (56%) of the reactors and 5 (33%) of the non-reactors. It is apparent that the skin test has value in an infected herd. If the reactors can be removed, a reduction in the dissemination of the disease might be expected.

Fecal Smears and Mucosal Scrapings The finding of small typical acid-fast organisms, especially in clumps, gives the most accurate diagnosis in clinical cases. However, one problem arises. In order to find the organism in a scraping, the disease must have progressed to a point in the colon where the diagnostician can reach a portion of infected mucosa; thus, this method is suitable only in terminal cases.

Fecal examinations have been used for a long time as a confirmatory test in clinical Johne's disease. Doyle (1956) reported

that only 25 to 30 per cent of the cases were positive at first examination. Cunningham and Gilmour (1959) described a method of preparing thin fecal smears for examination which makes it easier to find the organism.

In both fecal smears and mucosal scrapings, the finding of a single organism cannot be considered significant, but the finding of many organisms or of a clump of organisms is considered positive.

Serology The complement-fixation test (CF) has been used extensively in the past 10 years. There are two different types of antigens in common use. Both are described by Sigurdsson (1956). One is an extract of heavily infected mucosa used by Sigurdsson; the other is a suspension of artificially cultivated organisms used by Hole.

In cattle known to be infected and showing clinical symptoms, Hole (1958) reported that 94% gave positive reactions to the CF test. On farms where there was no history of the existence of the disease, between 10-20% reacted to the antigen (Rankin, 1958), (Chandler, 1955), (Hole, 1958), and (Van der Schaaf, 1955). In infected herds the complement-fixation test was positive in 40% of the animals (Rankin, 1958). Larsen and Vardaman (1958) studied an infected herd of 190 cattle in which 14 of 34 reactors to the CF test and 1 of 5 non-reactors harbored acid-fast organisms. This is the only report in the literature of a study on a naturally occurring infection in which skin reactivity, serology and post mortem results are correlated.

The final assessment of the complement-fixation test has not been made, but this statement by Sigurdsson (1956) best summarizes the situation: "With the antigen preparations now available it may be said that the practical usefulness of the complement-fixation test in any given animal population depends not only on the accuracy of the test as such, but also on the immunological experience of that population with antigenically similar materials presumably originating from related infection."

The Middlebrook-Dubos hemagglutination (HA) reaction and a hemolytic modi-

fication of this reaction have been described by Larsen *et al.* (1953), and Larsen and Vardaman (1958). In the same herd as previously discussed, these investigators found 14 out of 32 reactors to the HA test and 1 of 2 non-reactors also harbored acid-fast organisms in their intestines.

The evidence seems to indicate that most animals infected with Johne's disease will react to both the CF and HA test.

Demonstration The most accurate method of diagnosing Johne's disease is demonstration of the organism. This has been described in the section on fecal smears and mucosal scrapings. However, at post mortem this task is rendered less tedious. Scrapings of the mucoa around the ileocecal valve are stained by Ziehl-Nelson technique and examined. But, the organism may be so scanty that a concentration technique must be employed to find the organism. Such a technique using trypsin is described by Larsen and Merkal (1961). Histopathological section can also be used to demonstrate the organism. Demonstration of the organism is the only technique that assures complete accuracy in the diagnosis of Johne's disease.

Culturing of the organism is not usually attempted. Even the most experienced bacteriologist finds it difficult to grow the Johne's bacillus unless he does it routinely. Eight weeks is required for growth of the organism which makes culturing impractical for rapid diagnosis.

Host

Besides cattle, the other natural hosts of the Johne's bacillus are sheep and goats. Johne's disease has also been found in wild ruminants kept in captivity (Hole, 1958). Katic (1961) believes wild deer may serve as a reservoir and spread the disease from farm to farm.

Eveleth and Gifford (1943) found swine, horses and mules sensitive to the intradermal johnin test and found acid-fast organisms in some swine and horses, but not in mules. However, they did not find any clinical disease in these animals. Smith (1954) isolated an organism from the mesenteric lymph node of a horse and Rankin (1956) identified it as *Mycobacterium paratuberculosis*.

tuberculosis. This organism also experimentally produced the disease in calves.

Eveleth *et al.* (1942) discussed porcine Johne's disease. Smith (1954) examined 100 swine but did not isolate any Johne's organisms. Runnells (1955) reported that a pig had diarrhea and was in poor condition. At post mortem a thickened corrugated mucosa was observed from which acid-fast organisms were seen in tissue sections. No culture was made. To the epidemiologist, Johne's disease is primarily one of the ruminant. Though the organism has been demonstrated in the horse, this animal apparently serves only as a dead end host and does not present an active source of the infection.

Age Susceptibility

The disease as a clinical entity is most common in animals from 2 to 5 years of age, but infection usually occurs in calf-hood. Actually very little is known about natural infection (Hole, 1955). The one indisputable fact is that the organism has been found in the fetus, indicating congenital infection (Lawrence, 1956), (Doyle, 1958). Animals of any age can be infected experimentally. Doyle (1953) infected cows at the age of 5 years and produced clinical signs, but indicated that probably very few adults will naturally develop clinical infection if exposed as adults.

Transmission

Johne's disease is an enteric disease of the adult and the organism is excreted in the feces. The calf is infected naturally by ingestion of feces containing the organisms which have contaminated the water trough, feed bunk or udder of the dam. Recent evidence has indicated that another route also may be common. This is the transplacental route causing fetal infection. Lawrence (1956) discussed this matter and suggested that a bacteremia would have to be present. Since Doyle (1958) also found congenital infection, there is no reason to doubt the validity. If the incidence of fetal infection is greater than known, it could play havoc with

any control program. For example, a pregnant cow in a preclinical stage of Johne's disease could infect her calf and both would carry the organism before anyone would detect it.

The primary method of dissemination is traffic of infected animals from herd to herd. In many cases the method of introduction is not known, but there is sufficient information to indicate that newly acquired animals may appear in the herd prior to appearance of the disease (Larsen, 1955). Larsen also suspected drainage from an infected premise as being the source of infection.

Distribution

The disease is worldwide in distribution. There have been reports of the disease occurring in low lying wet areas and under acid soil conditions (Downham, 1951). Unpublished research conducted in Alabama indicated that Johne's disease occurs on all soil types (Larsen, 1962). The disease will occur wherever the environment has been contaminated with the organism. A good example is what happened in Iceland, (Gislason, 1956). Ovine Johne's disease was introduced into the Island in 1934 and the first case of bovine Johne's disease was diagnosed in 1945. In 1954, the area where bovine Johne's disease occurred was identical to the area where it was most prevalent in sheep.

Management also plays a role in the spread of the disease. The disease is associated with conditions where calves are raised with the dam or on an old nurse cow. The farmer may have a Grade A dairy but he may raise his calves in an old shed that has a foot of manure on the floor. Any type of management that allows a susceptible calf to come in contact with the contaminated feces will perpetuate the disease.

Breakdown

The first sign a farmer has that a clinical case of Johne's disease has developed is when the animal begins to lose condition, drop in milk production and scour. Once the disease is diagnosed the ques-

tion always arises as to what precipitated the clinical breakdown. Hole (1958) says that several factors may cause the breakdown — calving, low nutritional plane, heavy milk yield, parasitism and any other condition which will lower resistance. Recently this writer has seen two cases of Johne's disease following severe Iowa weather. This points to several predisposing factors all of which cause stress.

Summary

1. Johne's disease is caused by *Mycobacterium paratuberculosis*, an acid-fast organism, which requires a growth factor for culture. The organism is killed by sodium orthophenylphenate.
2. Diagnosis is based on clinical signs, skin reactivity, serology, and demonstration of the organism.
3. The natural hosts are ruminants.
4. Clinical cases usually occur in animals from 2 to 5 years of age.
5. Calves become infected by ingestion of the organism or by *in utero* infection.
6. The disease is worldwide in distribution with no relationship to any special environment. The type of management, however, may perpetuate the disease once it is established on a premise.
7. Various stress factors may cause a breakdown.

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Pitman-Moore Trip

The highlight of the Junior year, and perhaps the highlight of four years in these hallowed halls (other than receiving that certain piece of paper from the University) is the annual trip by the Junior class to the Pitman-Moore Laboratories in Indianapolis, Indiana.

By the time most of us piled out of the bus some 500 miles and 12 to 13 hours from Ames, we were so stiff and sore that we wondered whether it was all worth it. However, after a good night's sleep and a hearty breakfast under our belts, our apprehensions soon departed. The first morning was spent touring the biological laboratories at Zionsville, and the afternoon was occupied by a tour of their research Laboratory, also in Zionsville. The evening ended with a fine dinner and entertainment at LaRue's Supper Club.

The second morning consisted of recovering from the previous evening, and a tour of the central offices and distribution and processing plant located within the city. After an exchange of farewells and thanks from both parties, we left Indianapolis and stopped in Lafayette, Indiana. Here we visited the recently established school of Veterinary Medicine at Purdue University. The final leg of the journey ended in Ames, after a brief stop in Chicago.

In summary, it was whirl-wind tour that was both educational and entertaining to all in attendance. We heartily recommend to next year's junior class that they take advantage of this truly rewarding trip to Indianapolis, Indiana, through the courtesy of Pitman-Moore.