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Paratuberculosis

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PARATUBERCULOSIS

by

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PARATUBERCULOSIS (JOHNE'S DISEASE)

Paratuberculosis is a chronic and contagious infection of ruminants in which the most common finding is an enteritis. Its infectious nature was suspected at the beginning of this century and has since been confirmed (10). It is caused by an acid-alcohol-fast bacillus commonly called Johne's bacillus *(Mycobacterium johnei)* (44).

GEOGRAPHIC DISTRIBUTION

Paratuberculosis is found in nearly all areas that ruminants inhabit. The disease has recently been reported to be of significance in the United States of America (52), Great Britain (10), Denmark (22), Pakistan (24), the U.S.S.R. (43), Iceland 37), Turkey (13), Madagascar (7), Canada (32), Japan (35), and Ireland (38, 39, 41). It is most evident in areas where intensive agriculture is practiced and where continued close confinement of stock occurs.

CAUSAL AGENT

It was in 1895 that Johne and Frothingham discovered, for the first time, an acid-alcohol-resistant bacillus in the intestine of a bovine animal which was affected with a chronic diarrhea. For some years the organism was confused with the avian tubercle bacillus (25, 44).

Later, McFadyean (1907), in England, described the disease in detail. Twort (1910) and Twort and Ingram (1913) obtained a pure culture of the organism and gave a general description of the disease.

Mycobacterium johnei is a short, thin rod five-tenths microns wide by one to two microns in length. It is nonmotile and is considered to be gram-positive.

In smears of the intestinal mucous membrane, the lymph nodes, and feces, the organisms occur in groups, but may also occur as single, separated organisms (2). Johne's bacillus is acid-alcohol-fast, is more pleomorphic than the tubercle bacillus, and stains evenly by the Ziehl-Neelsen method. Fluorescent microscopy may be used. Non-acid fast forms are described (44).

The slowly growing bacillus isolated from the bovine is the type species; two more slowly growing strains have been isolated from sheep (44).

CULTURE AND PRIMARY ISOLATION

The organism will grow in an artificial medium if it contains an "essential substance" which is indispensible to its metabolism (25). The current source of this substance (mycobactin) is a culture of *Mycobacterium phlei,* glycerinated and killed by heat 44). Unanimity in recommending the most useful medium for primary culture is not seen, but all agree that this growth factor must be present (25, 44, 47).

It is noted that the length of time required for a culture to grow (2-3 months) is too great for this method to be considered as a useful diagnostic aid (2).

Studies have been conducted to devise an improved technique for primary isolation of *Mycobacterium johnei.* Trypsin digestion of intestinal mucosa was followed by decontamination with 1 N so-

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dium hydroxide; this was effective in preparing the inoculum for primary cultivation. A lymph node-egg yolk medium was considered superior to several other media for primary cultivation and subcultivation of newly isolated strains (27, 31).

RESISTANCE TO CHEMICAL AND BIOLOGICAL PRODUCTS

The resistance of *Mycobacterium johnei* to heat and to chemical disinfectants resembles that of the tubercle bacillus.

The organism, which is an obligatory parasite, under favorable conditions may survive outside the animal body for a long time. *Myco. johnei* can remain viable in the feces for more than eleven months, in bovine urine for seven days, in sterile water for nine months, and in non-sterile river water for 160 days (44). It may survive in saline and tap water for 17 months, in the dry state for 47 months, may survive temperatures of from —14 to 38 degrees Centigrade, and may survive in sunlight for over 65 hours (25).

The following substances prevent growth: cresol compounds, 1:64; phenol, 1:40; orthophenylphenate, 1:100; alcohol, 95%; bicholoride of mercury, 1:100; calcium hypochlorite, 1:50; and methylene blue, 1:500 (44).

In vitro studies of antimicrobial agents have involved antibiotics, synthetic antituberculosis compounds, antimalarial agents and related compounds, sulfonamide derivatives, and miscellaneous compounds. Antibiotics were noted to have some effect as did the sulfonamides but, as the studies were in vitro, field applications would not necessarily render like results (15). Wide gaps exist in the knowledge of the resistance of the organism and in the treatability of the disease (47). The disease is still considered incurable.

SUSCEPTIBLE SPECIES: USE OF LABORATORY ANIMALS

Animals naturally susceptible to Johne's disease are most commonly the ruminants (cattle, sheep, and goats) (5, 19, 23, 24, 25, 44, 47, 56, 57). In the deer family, the roe deer, Japanese deer, red deer, and reindeer have been reported as susceptible to or carriers of the disease (22, 23). The antelope and gnu are reported as susceptible as are the llama and camel (23, 25, 43, 47). The organism has been isolated from the pig and from the horse, but such isolations are considered to be of little significance (25, 45, 47).

Research personnel have attempted utilizing various laboratory animals to effect a more complete study of the disease process induced by *Mycobacterium johnei.* Natural disease (like that in cattle and sheep) was not reproduced in hamsters, but the similarities were considered significant (18). The ease of infection makes the hamster a valuable laboratory animal.

Swiss white mice are of no use when attempting to study the classical intestinal Johne's disease (8). These mice did show some protection due to vaccination in another experiment but there were no clear cut results (10). The use of these mice is not recommended. Another comparative study of several types of mice did reveal a type (C_{57}) which offered some promise (11, 12).

Experiments using white rats (wistar) have been conducted. Liver and spleen lesions were evident but were not serious; no intestinal infections were noticed (9). Rats are considered to be of little value in the study of Johne's disease.

INCIDENCE AND ECONOMIC LOSS

It is difficult to obtain accurate figures to depict the true incidence of Johne's disease. In England, the organism *(Myco. johnei)* is recovered from seven to seventeen per cent of the cattle and from about twenty-one per cent of the sheep (47). The incidence of clinical disease is considerably less. In Pakistan 27% of the cattle are infected as are 17% of the sheep (24). Paratuberculosis was first noticed in Iceland in 1933, when it was imported. Losses rose to eight to nine per cent annually. Over 100,000 sheep were lost before a potent vaccine was introduced (37).

Loss from clinical disease can be fairly well delineated; loss from non-clinical disease (lowered production, etc.) is unknown. There is no salvage value obtainable from a clinical case.

TRANSMISSION

The transfer of Johne's disease from animal to animal may occur in various ways. The most common method is via continuous ingestion of contaminated feces or of contaminated fomites (25). The young are commonly more susceptible to infection even though the disease is clinically manifested in adults (43, 44). Of adult cattle maintained in an infectious environment, 14% later showed clinical signs, while another 67% were found to be carriers (50). Calves maintained in an infectious environment developed the clinical form of the disease in 67% of the cases (49). Resistance is thought to occur at about six months (43, 49). Intra-uterine or congenital infections are known to occur (25, 30). Although the organism can be isolated from the milk, it is not commonly found there. Thus, the milk would not be a common source of infection for the young animal, but a teat contaminated with feces containing the organism would be more probable (53). Experimental infection via parenteral routes can result in clinical disease, but more commonly results in the normal carrier animal (25, 44, 48).

Introduction into areas or countries previously free of the disease is most commonly through the non-clinical carrier animal (37). Wild ruminants are also a source of transmission (23). Drainage water from infected areas may carry the organism (25).

PATHOGENESIS: CLINICAL SIGNS

It is generally felt that Johne's disease is essentially an infection acquired by the young calves and that several years pass before the disease develops, or the infection remains latent until physiological or other conditions arise for its development (43, 44).

It has been known for a long time that a bovine may be infected without showing any clinical signs and that sometimes these animals which appear to be healthy during life show extensive lesions at post mortem examination; and, further, that infected animals may be carriers of the organism for some years without the occurrence of clinical symptoms. There is no relationship between the severity of the clinical signs and severity of the lesions or the numbers of the organism present.

Certain factors have been incriminated in causing the development of the clinical disease. There are different conditions which can activate a latent infection. They include calving, malnutrition, high production, parasitic infestations, mineral deficient soils, low lying pastures, and acid soils (25, 43, 44).

An early sign of approaching breakdown is the appearance of a swelling under the jaw. This is by no means a constant symptom, but its relationship to Johne's disease has been recognized for many years. The swelling is edematous in nature and frequently disappears when diarrhea (dehydration) begins. A sharp fall in milk yield occurs with impending Johne's disease. An unexplained loss in flesh occurs; the skin becomes tight (dehydrated); the hair coat becomes rough and discolored (24, 25, 39, 47).

In the early stages the diarrhea tends to be intermittent and can be controlled by astringent medication or by housing the animal and feeding dry feeds. The diarrhea becomes more protracted and is not controllable by medication. During these attacks of diarrhea, the feces are passed in a steady stream with no signs of straining. They may be of a fluid consistency and are always dark in color and homogenous in texture. Small bubbles are usually seen on the surface of the feces for a short time after it has been dropped. A light-colored watery fecal sample containing easily visible particulate matter in suspension is not likely to be a case of Johne's disease (47).

In sheep and goats, diarrhea is not a constant sign, but the feces often loses its pellet formation for short periods. Loosening of the wool has been reported as a symptom (non-specific) in sheep. The signs and pathomorphological changes in other more exotic animals are similar to those in cattle. The only constant symptom present in all animals is progressive emaciation. The appetite usually remains good in all species unless secondary complications occur (23, 39, 47).

Once signs (overt) have developed, death is the end result. For all practical purposes it should be assumed that once the disease can be diagnosed with certainty, the animal is beyond recovery.

LESIONS: CATTLE

The alimentary tract is the main site of lesions in Johne's disease, and in advanced, chronic cases, these may extend from the duodenum to the rectum. In such cases, the characteristic cerebrum-like corrugations of the thickened mucous membrane are exposed upon opening of the intestine. In less severe cases the lesions are not so marked and are unevenly distributed. The most commonly affected portion of the tract is the terminus of the ileum (ileo-cecal junction).

Although petechiae on the crests of the ridges forming the convolutions may give the mucous membrane a hemorrhagic appearance, hemorrhage is not a characteristic feature of the disease and the surface of the mucous membrane remains unbroken. Necrosis, caseation, and calcification are not seen in cattle.

The lesions are not always easily recognized grossly and histological examination may be necessary. Histologically, the intestinal lesion may vary in severity from a few groups of endothelioid cells to a symplasma in which the villous structure is completely lost in a confluent mass of endothelioid cells. Microscopic lesions may be seen in the lymph nodes of infected cattle. They are usually situated just beneath the capsule and consist of aggregates of endothelioid cells and an occasional giant cell. These lesions are similar to early tubercular lesions but usually don't become calcified, encapsulated, caseous, or necrotic (in cattle at least) (25, 44, 47, 52).

Naturally occurring arteriosclerotic lesions have been found in cattle affected with Johne's disease. A high percentage (67.7%) of those cattle condemned for Johne's disease had gross arteriosclerotic lesions. Fibrotic intimal and endocardial thickening, plaque formations, and calcifications of the large vessels were the most characteristic lesions. A direct relationship was observed between arteriosclerotic lesions and macroscopic intestinal lesions. The lesions appear to be self limiting—even though they probably result from the metabolic disturbance occasioned by the infection (1).

LESIONS: SHEEP

Primary lesions in sheep as a result of *Mycobacterium johne's* infection occur in the lymph follicles of the intestinal wall (Peyer's patches). Thereafter contiguous spread occurs (3, 5, 36). Necrosis, calcification, and caseation of the lymph nodes may be present in sheep and goats. In sheep, the intestine may be pigmented an intense yellow-orange, but there is often little thickening or corrugation to be seen. The presence of Johne's disease in cattle associated with infected sheep may be seen but doesn't necessarily occur (44, 47).

DIAGNOSIS

It is most common to attempt diagnosis of paratuberculosis by utilizing the history, clinical signs, and lesions as just described (2, 13, 24, 25, 39).

Laboratory tests may also be used to diagnose the disease. These tests are essentially aids in indicating evidence of infection. They often have a different significance at different stages of the disease.

The feces may be examined for evidence of the organism or scrapings of the rectal mucosa may be used. The finding of acid-fast bacilli in clumps in either of these cases confirms a diagnosis of Johne's infection (3). It should be noted that false negative samples may be found in up to 70% of the cases (2, 13, 24, 25, 40, 55).

Culture tests may also be used in diagnosing paratuberculosis, but the long period required for growth usually reduces the value of culturing and it is seldom utilized (2, 13, 25). Two to three months are required for growth and a negative result doesn't nullify a positive diagnosis (47).

Allergic reactions for the diagnosis of Johne's disease have been used in the past but are not much in vogue at present. Cows with Johne's disease have a varied response to no response while cows free

of the disease may react to the allergin. As animals with the infection approach the clinical stage of the disease the reaction to intradermal johnin injection is often reduced or absent (2, 13, 24, 25, 28, 32, 39, 40, 41, 44, 47, 55). The allergic test is not reliable in zoo (exotic) animals (23).

Of the serologic tests, both the agglutination and the complement-fixation have been used, but only the complement-fixation test is common now. The problem of cross reaction with antibodies to mammalian tuberculosis can be avoided by using the test only in tuberculosis free herds. The complement-fixation test will be positive in 95% of cattle with clinical disease or showing extensive intestinal lesions post mortem; this is very good but cattle harboring *Mycobacterium johnei* in lymphatic tissue but without intestinal lesions will often not react in the complement fixation test and often ten to twenty per cent of cattle in herds free of Johne's disease will give a positive result. Calves may react to the complement-fixation test because of absorbed maternal antibodies. The complement-fixation test is regarded by many as being of main value in confirming the diagnosis of clinical infections (2, 7, 13, 16, 19, 25, 29, 32, 40, 41, 42, 44, 46, 47, 55).

It is of interest to note that the two reactions (*i.e.* the allergic response and the presence of complement-fixing antibodies) seem to be quite independent of each other and may be the result of two different antibodies. Nearly all cattle infected with *Mycobacterium johnei* will give a positive reaction to avian tuberculin or to johin at sometime during the course of the infection. The reaction usually peaks a month or so after the inoculation and thereafter fluctuates between some degree of reaction and no reaction until intestinal lesions become extensive when the reaction is likely to be minimal. Conversely, the complement-fixation test develops much later, and, although fluctuations do occur, it becomes more certainly positive as the intestinal lesions become more advanced (Ibid, previous paragraph).

The ultimate diagnosis is the result of post mortem examination and our concern here need be only with the intestine and its associated lymph nodes. The carcass will show a varying degree of emaciation and amount of abdominal fat. The mesenteric lymph nodes are said to be swollen and edematous. The lymph nodes are a good source of material for microscopic and for cultural examination.

Intestinal corrugations that do not leave when the intestine is stretched are quite diagnostic for Johne's disease. In all cases of doubt, a smear of the mucous membrane, stained by the Ziehl-Neelson method, should be examined for the presence of acid-fast bacilli. If this fails, a histological examination of the intestine, especially near the ileum termination, should be conducted (47).

PROPHYLAXIS

The results of vaccination programs indicate a significant degree of protection (4, 6, 14, 17, 20, 21, 37, 51, 54). It is not certain that the use of the vaccine will not interfere with the correct interpretation of the tuberculin test (14, 17, 20, 21, 26, 33, 34, 42, 54). Until more is known about the vaccines used, they are being restricted because of possible interference with the tuberculin test (24, 43, 47). A vaccine which will not produce skin sensitivity is being sought.

Simple hygienic measures may have a marked effect in reducing the effect of clinical Johne's disease. Heavy culling of poor producers should be practiced. At one time the immediate removal of the calf from direct and indirect contact with adult cattle was thought to be a solution to the problem; unexplained breakdowns occurred in herds where this was practiced. These may have been the result of intra-uterine infection; calves born from cows showing clinical signs of the disease or which show clinical signs shortly after infection should not be raised.

THERAPY

Paratuberculosis is still on the list of incurable diseases. Many substances have been tried but none have shown significant promise under field conditions (15).

APERCU

The disease, paratuberculosis, caused by *Mycobacterium johnei,* has been discussed. The eradication of many other diseases has caused this yet incurable one to assume greater importance than has previously been evident. The problem of Johne's disease is yet unsolved; wide gaps exist in the knowledge about it.

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Christmas Party

The annual Jr. A. V. M. A. Christmas Party was held on Dec. 16, 1964 at Great Hall Memorial Union. Attending the party which was put on by the Junior Class, were instructors, students, their wives, and children. Under the direction of Masters of Ceremonies, George Atkins and Loren Appell, the guests enjoyed winning a variety of door prizes donated by Ames merchants and several pharmaceutical companies. Entertainment was provided by a quartet which included Dr. Howard, Dr. Packer, Dr. Burt, and Mr. Engen. A skit was presented by members of the Junior Class and Christmas music was offered by the Veterinary Chorus directed by Wayne Fawver. At the conclusion of a most enjoyable evening, the children enjoyed a timely visit from Santa Claus who had treats for all.

Alpha Zeta

The Fraternity of Alpha Zeta, an honorary agriculture fraternity, has initiated nine new members from the College of Veterinary Medicine into it's organization. Three junior veterinary students have also transferred their membership to the Wilson Chapter of Alpha Zeta here at Iowa State.

Seven of the new pledges are from the Junior class and include: George Atkins, Mervyn Daehler, Larry Mahr, Dave Olson, Daniel Stoner, James Van Buren, and Bruce Van Zee. Myron Hinrichs, Bill Wilson, and Gerald Orlando, who have transferred their membership to the Wilson Chapter at Iowa State are also in the Junior Class in Veterinary Medicine. The two other initiates are Jack Hayes, a Senior, and Al Metz, a sophomore in the veterinary medicine curriculum.

Wilson Chapter of Alpha Zeta Fraternity was founded at Iowa State College on November 27, 1905, by P. A. Campbell and W. H. Pew. The objectives of the chapter, and of the fraternity as a whole, are to foster high standards of scholarship, character, and leadership and by doing so to render service to the students of agriculture and promote the profession on the whole.

Members for Alpha Zeta are selected from among undergraduate and graduate agriculture students of high scholarship on the basis of character, leadership and personality. The student must have completed at least one and one-half (1½) academic years of his college course and his grade average must place him in the upper two-fifths of his class.

We in the veterinary college wish to extend our congratulations to the men who have proven their abilities and have been honored in Alpha Zeta Honorary Agriculture Fraternity.