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Johne's Disease

A Transmissible Disease of Cattle

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In 1915 the Wisconsin Experiment Station began a study of Johne's disease which has been continued without interruption to the present time. In addition to presenting the results secured in this study, it is the purpose of this publication to set forth the present state of our knowledge concerning Johne's disease by reviewing in some detail the publications of other workers on the same subject.

Thus far the disease has attracted little attention in this country on the part of practicing veterinarians or cattle owners. Recently, however, the Federal Government has recognized it as a disease to be fought along the same lines as bovine tuberculosis, and has provided funds for the indemnification of owners of affected cattle. The Federal money becomes available in any state only when the state has made an appropriation for the same purpose. As a result of this Federal action, the disease is sure to become more widely recognized. It may be that such a review of the subject as is here presented may prove of timely interest.

For the benefit of those who wish to pursue the subject further, the list of references has been made as complete as possible. Not all the original papers listed have been consulted, nor are the individual references complete in all cases; however, it is believed that what has been included here will provide a useful and reliable source of information for those interested in the study of Johne's disease. The monograph of F. W. Twort and G. L. Y. Ingram (1913) is the most important publication dealing with Johne's disease.

Definite and conclusive statements concerning many phases of the disease cannot be made at this time. Our knowledge of it is still fragmentary and will be so for many years. Aside from the fact that it is more difficult to study than is tuberculosis, the gathering of information concerning it has been limited by the general failure to recognize its importance, which has resulted in a lack of interest on the part of most investigators.

The study of tuberculosis has engaged the attention of thousands of students for nearly fifty years, and has left many questions unanswered. On the other hand the study of Johne's disease has occupied perhaps a score of workers for twenty years, and has presented difficulties in cultural and animal work far greater than are ever encountered in the study of tuberculosis. It is not surprising, in view of these facts, that more progress has not been made in the knowledge of Johne's disease; and it is to be hoped that the present increasing interest will result in a more intensive study of the disease by a larger number of investigators.

The publications which we have attempted to review contain many contradictory statements, which have been interpreted with the understanding that the interpretation will need revision in many respects as our knowledge concerning the disease increases.

Names Given the Disease

Many names have been applied to the condition which is most widely and most appropriately known as Johne's disease. A number of the suggested names are not suitable because, though descriptive, they are not specific. Thus "para-tuberculosis," which suggests the similarity of the causal organism to the bacillus of tuberculosis, has been used to refer to other diseases. The names chronic bacterial dysentery, chronic enteritis, and chronic pseudo-tuberculous enteritis cannot properly be used to designate a disease caused by a specific organism, as is true in the case of Johne's disease.

In Switzerland the disease has been called "Kaltbrändigkeit," thirst without fever; in Denmark the term "Laaland Disease," is sometimes used, due to its prevalence on the island of Laaland. "Scrapy" or "scrapie" is a name used in Scotland and England to refer to that which is supposed to be Johne's disease in sheep. It seems probable that a number of diseases, among them Johne's, are included under this popular term, or else that the affection is due to other causes than the Johne's bacillus (Gaiger, 1924, Twort and Ingram, 1913).

The extreme emaciation which marks advanced stages of the disease gave rise to the popular terms "wasters" and "piners" in England and "canners" in America. It is probable that Johne's disease is a minor cause of the condition reflected by these words.

The name Johne is properly connected with the disease, since it was he who, in 1985, first demonstrated the causal organism. Together with Frothingham, an American student, he described acid-fast bacilli found by them in the intestine of a cow which had reacted to tuberculin and which showed no lesions on post-mortem examination. Johne and Frothingham believed from the microscopic appearance of the organisms that they were avian tubercle bacilli. Not until eleven years later was Johne's disease differentiated from tuberculosis (B. Bang, 1906).

Characterization of the Disease

Johne's disease is a specific chronic enteritis of cattle caused by a member of the group of mycobacteria or acid-fasts, to which the tubercle bacillus also belongs. The causal organism grows in the mucous membrane of the intestines and in the mesenteric lymph nodes. It produces a diffuse thickening of the bowel wall which interferes with the absorption of nutrients and of water. The disease is marked by intermittent diarrhea and by emaciation.

Primarily a disease of cattle, it is also found in sheep. Its importance in the sheep industry cannot be estimated because it has been confused with other diseases of sheep. The infection has not been reported in any other species of domestic animal or in ruminants in captivity with the exception of one case in a deer reported by M'Faydean (Twort & Ingram, 1913).

Distribution of the Disease in the World

England, Holland and Switzerland, three of the world's chief sources of dairy cattle, have apparently harbored for a considerable time herds

infected with Johne's disease. Countries importing cattle from these sources evidently import the disease with them, since outbreaks are never met in native herds but only in those which include animals of imported breeds.

Twort and Ingram believe that records show its presence in England in the first half of the 19th century. It seems to have been present in Holland for many years. Marcus (1904) reports that Koorevaar, a Dutch veterinarian, had noted the thickening of the intestinal wall of emaciated cattle and that in some districts the name "Scheisser" was used as a probable synonym of "scourer."

The importation of various breeds from the countries mentioned has given an opportunity for the introduction into the United States of Johne's disease. It has been reported from South Africa and from India as well,

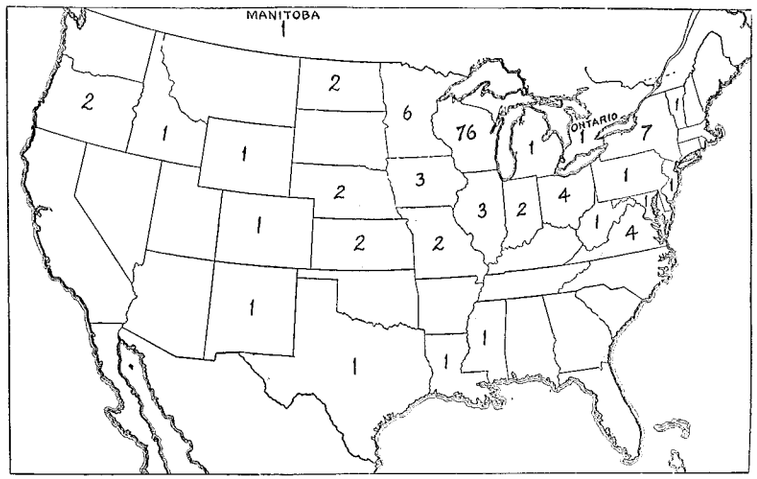

FIG. 1.—THE DISTRIBUTION OF JOHNE'S DISEASE IN THE UNITED STATES AND CANADA

The numerals refer to the number of herds reported to us as infected with the disease.

and is probably present in all regions to which the English, Dutch and Swiss breeds have been sent. Our quarantine authorities have never considered it directly. Due to the lack of a means for detecting it, other than by clinical symptoms, it is not probable that much could have been done to prevent the entrance of incipient cases. A prominent importer of cattle from the Channel Islands has stated that animals have died in our quarantine stations from what was apparently Johne's disease. It is obvious that it is as important to prevent the importation of animals affected with Johne's disease as it is to prevent the entrance of tuberculous animals. Since a diagnostic agent is now available, cattle should be tested at the point of export in order to prevent the further introduction of the disease into this country.

Once introduced into a country it spreads imperceptibly, first because veterinarians are not on the lookout for it, and second because of its

chronic nature. The efforts which may be made to eradicate Johne's disease in this country in the herds in which it now exists will be lessened in effect if more cases are constantly being imported from abroad.

The disease is not limited to any breed in particular, though it has been reported as occurring so frequently in cattle of the Jersey breed that the inclination is to consider all other breeds resistant. It has been observed in Devon, Shorthorn, Herford, Guernsey, Brown Swiss and Holstein cattle. We have encountered it more frequently in herds of the Channel Island cattle than in those of other breeds. Its prevalence depends on opportunity for infection, rather than on the susceptibility of the different breeds.

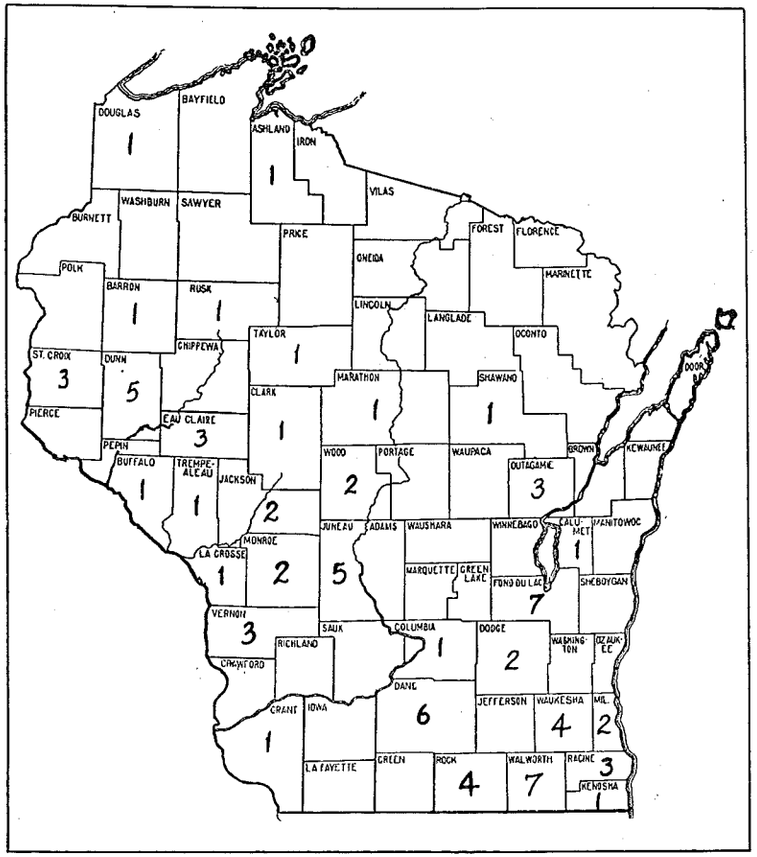

FIG. 2.—THE DISTRIBUTION OF JOHNE'S DISEASE IN WISCONSIN
The number of cases reported from each county is expressed by the numerals.

Distribution of the Disease in this Country

It seems probable that Johne's disease is widely distributed in the United States. Reports regarding it have been received from 27 states as presented on the accompanying map, Fig. 1. The herds belonging to the Colleges of Agriculture in eight of these states have been or are infected.

In Wisconsin 76 infected herds have been brought to our attention. The distribution is presented in Figure 2.

Prevalence of the Disease

Nothing definite can be said concerning the prevalence of the disease in the various sections in which it is known to exist. It is certain that a very small percentage of the infected herds is known. The only cases which have been recognized are those in herds in which the losses have been excessive, or in herds belonging to especially alert farmers, or cared for by veterinarians who have had the disease in mind as a possible factor in explaining losses from the herd Meat inspectors, veterinarians and leaders in live stock sanitation have been largely oblivious of its presence, until it has been brought to their attention by the farmers. This condition should be reversed and, no doubt, will be within a short time.

The disease has, at present, a limited number of sources from which it can spread. These sources are largely the herds of pure bred cattle, especially those of the Channel Island breeds. These sources of infection will continually increase, unless agencies are operative to offset the constantly increasing commerce in cattle from such herds. Dr. V. A. Moore (1924) has compared the present position of Johne's disease to that of bovine tuberculosis sixty years ago and has prophesied that, if not controlled, it may become a more troublesome scourge for future generations than tuberculosis is for the present generation of cattle owners.

There is no need for worry concerning the disease. It certainly spreads no more rapidly than tuberculosis, and probably less so. The relatively low percentage of bovine tuberculosis in important dairying districts, such as central and northern Wisconsin, indicates that bovine tuberculosis requires many years to become very prevalent. Johne's disease seems to exemplify a condition to which the old adage, "a stitch in time saves nine," may be applied.

Table I.—Losses from Johne's Disease in a Number of Wisconsin Herds

Herd	Number in herd	Duration of infection	Number removed because of infection	Yearly losses
		Years		Per cent
1	45	8	30	8.5
2	50	17	41	4.7
3	40	15	20	2.2
4	35	10	22	6.2
5	18	10	22	12.0

Losses Occasioned by the Disease

The losses which may come to the owner of a herd infected with Johne's disease is a matter quite apart from its regional distribution and its prevalence. Table I gives the losses from five herds that have come under our observation.

Recently Ernest (1927) has reported on a herd of 207 animals from which 27 cows had been removed on account of this disease during 18 months. After this, a test was made and nearly one-half of the remainder was found to be infected.

A few reports from foreign countries regarding the extent of infection in individual herds are presented. These, while perhaps not of immediate importance to readers of this publication, are of interest in that they indicate to what extent losses from Johne's disease may occur.

Table II. was compiled from data gathered by O. Bang (1914).

Table II.—The Extent to Which Johne's Disease Was Found in Some Danish Herds

Herd	Number of animals over two years old	Infected Per cent	Number of animals under two years old	Infected Per cent
1	139	45.3	31	0.0
2	69	37.7	25	0.0
3	67	34.5	26	4.0
4	148	10.8	73	5.5
5	99	10.0	78	10.0
6	291	13.0	77	8.0
7	85	23.5	29	0.0
8	89	9.8	19	0.0

Avian tuberculin was used as the diagnostic agent on herds known to be free from tuberculosis as shown by testing with mammalian tuberculin. No data were presented as to the actual losses encountered in such seriously infected herds.

Table III. indicates what Krautstrunk (1918) found on testing with avian tuberculin.

Table III.—The Extent to Which Johne's Disease Was Found in Some German Herds

Herd	First test		Interval months	Second test	
	Number tested	Per cent reacting		Number tested	Per cent reacting
1	19	36	11	20	25
2	40	12	8	35	25
3	25	28	8	30	46
4	36	11	10	39	17
5	77	22	10	131	19

Bugge and Cordsen (1908) describe a herd of approximately 120 milk cows, besides the young animals from which 110 head had been lost in 15 years.

Edwards (1926) states that Johne's disease is a serious limiting factor in some important herds of southern India and that no measure for eradication is unduly harsh if it is desired that the herds should be kept together at all. He believes it is more important to free a herd from Johne's disease than from contagious abortion, considering the latter as a self-limiting disease.

Twort and Ingram (1913) state that in some areas in England the losses are greater from Johne's disease than from tuberculosis. They quote O. Bang as saying that from 29 Jersey herds in Denmark, 150 animals died in one year. No statement was made as to the size of the herds. O. Bang states that from a herd of 150 animals, 22 were removed in one year on account of Johne's disease.

The figures and statements quoted can not be considered typical of the losses caused by Johne's disease in most herds where it exists. The extreme cases are those brought into prominence. The data are, at the most, indications of possible losses, serious if they are allowed to continue, but preventable if taken in time. It would seem that the tax laid by Johne's disease on an infected herd is comparable to that imposed by bovine tuberculosis. In many herds, perhaps the great majority, the annual losses will not be large, in others they may endanger the economic success of the herd. In any case there seems to be no reason why the tax should be a continuous one, since Johne's disease is preventable.

Symptoms Shown by Infected Cattle

The period of incubation of Johne's disease is unbelievably extended in some cases. From observations made in England it seems that at least six months must elapse after invasion before symptoms become evident. This implies that the disease is not frequently noted in young animals, a fact which is evident from the data of Table II. The lack of symptoms in young animals does not, however, mean that such may not be infected. Ernest (1927) in testing a herd of 207 animals with johnin found nearly one-half of the animals responding to the test. Among those giving positive reactions were 17 calves ranging from three weeks to six months of age. No post-mortem data are available on the young animals.

Statements are frequently met which indicate that symptoms in young animals are exceedingly rare, as, for example, Edwards (1926) states that the affection is nearly always noted in adult cattle over two years old, probably due to the long period of incubation.

It is known that animals may harbor the infection for years and yet show no symptoms of the disease. Krautstrunk (1918) describes an animal which had been kept five years after reacting to the test for Johne's disease without showing clinical symptoms. Soon after calving the animal failed rapidly. A six year old cow developed Johne's disease from which she died three to four months later. At six months of age this animal had been purchased from a herd in which the disease was known to exist. As far as could be determined this was the only contact with Johne's disease this animal had ever had. There is, in the case of Johne's disease, as in tuberculosis, a tendency for the rapid progress of the disease in some

animals which had not previously shown symptoms thereof. Such decline frequently follows parturition. Wilkins (1926) states that affected animals may remain apparently healthy for at least two years, and some may never become visibly ill though suffering from the disease, a fact which must be taken into consideration in any effort to eliminate the disease by removal of animals showing clinical symptoms thereof.

O. Bang (1914) followed the history of 54 animals which had reacted to avian tuberculin. Five years later, eleven such animals were still apparently in perfect health. During the five-year period 43 animals had either been killed or had died; over one-half of these had never shown any physical symptoms. On post-mortem examination the supposedly causal organism was found microscopically in 86 per cent of the 43 examined.

Johne's disease is probably one of the most chronic of all those caused by bacteria. An animal may show unmistakable symptoms of the disease and remain alive for a number of years, exhibiting neither marked improvement nor decline. One of the animals under observation by us showed in April, 1925, what were then considered symptoms of a rather advanced case of the disease. There was little change during the subsequent two and one-half years. Positive reactions to johnin were obtained a number of times during the period. The animal died in November, 1927. Typical lesions were found and acid-fast bacilli were demonstrated in the tissues.

The most striking symptom is the gradual loss of flesh. This may continue until the animal becomes a mere skeleton, a condition well illustrated in some of the accompanying photographs. The eyes remain bright but become sunken, due to absence of intra-orbital fat. The muzzle remains moist. Commonly there is no fever and the appetite is not impaired. These conditions are very similar to those frequently noted in tuberculosis; the latter being the more common disease is likely to be the one thought to be present in the herd, and even non-reaction to tuberculin does not always relieve the suspicion of tuberculosis.

The other marked symptom is diarrhea, which appears and disappears to appear again sooner or later. Diarrhea is not a constant symptom of the disease, since well advanced cases sometimes show no evidence of this trouble. Some authors speak of the disagreeable odor of the feces during periods of diarrhea; still other authors state that no disagreeable odor was noted.

The most that can be said for the symptoms of Johne's disease is that marked emaciation and intermittent diarrhea should arouse the suspicion of the owner. Non-reaction to tuberculin increases the probability of Johne's disease being present. The final answer can be obtained only through a post-mortem examination or through the use of the specific diagnostic agent, johnin.

The exceedingly slow development of the disease and its ebb and flow in the animal are likely to lead to the conclusion that some physiological disturbance is the cause of the condition, rather than that an infectious disease is present. This lack of recognition of the nature of the trouble frequently gives opportunity for wide-spread infection of the herd.

Changes Produced in the Tissues

The characteristic lesion occurs in the intestine, the wall of which is thickened over a greater or smaller area. In cases of long standing there is a thickening of the jejuno-ileum extending 20 or more feet. Cases are reported of a thickening of the entire intestinal tract. Such a marked involvement is rare. Inflammation of the ileo-caecal valve is frequently observed. At times the valve is greatly swollen, becoming 15 to 20 times its

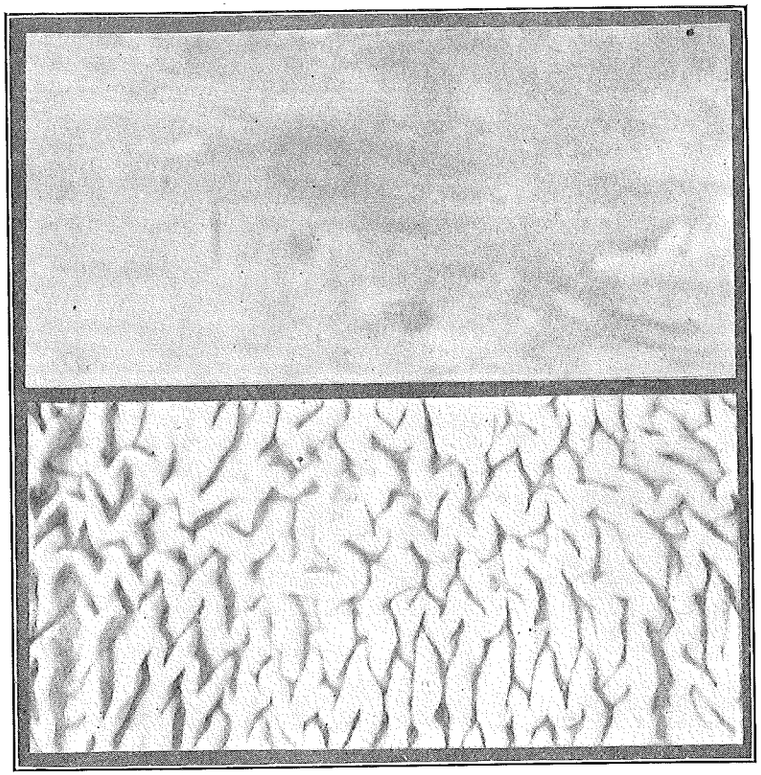

FIG. 3.—HEALTHY AND DISEASED INTESTINAL WALLS

The upper part of the picture represents a normal wall when tightly stretched, the bottom part the wall of an animal infected with Johne's bacillus. It is impossible to remove the wrinkles of the diseased wall by stretching it.

normal size. A piece of thickened bowel presents on its mucous surface a peculiar wrinkled appearance; the mucosa seems to be thrown into folds and ridges. Normal gut at times presents wrinkles which will, however, disappear on stretching. (Fig. 3.)

It is not uncommon for a piece of normal bowel to be interposed between two thickened pieces. This patchy tendency is frequently noted in cases

in which the caecum is involved. We have noted nothing characteristic as to the color of infected mucosa. Occasionally there may be small petechiae, irregular in outline and distribution. Twort and Ingram noted a characteristic pinkish yellow color. This yellow color does occur, but has not been constantly observed by us. At times the intense inflammation in the region of the valve will impart a dark red color. It is entirely possible that these acute inflammatory changes are due to the secondary invasion with other types of bacteria. Part of the reacting cattle slaughtered by us had never shown symptoms of Johne's disease. In several of these animals the ileo-cecal valve was markedly enlarged and inflamed. On the other hand in some of the cases of long standing inflammatory changes were slight, or entirely absent. We have never observed ulcers or nodules. The macroscopic changes of the lymphatic glands are meager and confined to a slight enlargement and softening of the substance. On section a serous fluid frequently exudes. In one case of long standing a marked induration was noted.

The tissue destruction, so characteristic of tuberculosis, is lacking in the case of Johne's disease. Frequently the lesions seem to bear no relation whatever to the physical condition of the animal. Indeed the changes in the intestinal wall, even in advanced cases, may be so slight as to pass undetected at a post-mortem examination unless one has Johne's disease clearly in mind while making such an examination. The lesions will not commonly be found unless the intestine is opened and the internal wall examined. Macroscopic evidence of the disease may be lacking, yet acid-fast bacilli may be demonstrated microscopically.

Microscopic Appearance of the Tissues

M'Faydean (1918) made an extensive comparative study of the microscopic pathology of the lesions of Johne's disease and of tuberculosis. He emphasized the fact that in the case of tuberculosis there seemed to be no limit to the invading powers of the bacilli, while with Johne's disease the invasion begins and ends with the intestines and their lymph glands.

In tuberculosis there is much tissue formation with ultimate necrotic and degenerative changes, while with Johne's disease there is little formation of new tissue. The nodules so characteristic of tuberculosis are absent. In the initial stages the intestinal lesions of Johne's disease have a histology absolutely identical with that of tuberculous lesions. In the later development of the lesions there is not to be noted the necrosis and caseation which is so characteristic of tuberculosis.

C. C. Twort (1914) believes that caseation occurs only on the death of the Johne's bacilli. It is never noted in the organs of naturally infected animals, but was noted by him in rabbits and guinea pigs which had been inoculated intraperitoneally, but which showed no evidence of the disease other than a number of caseous masses scattered throughout the abdominal cavity.

In the last stage of lesion formation giant cells are apparently formed by fusion and degeneration of the cytoplasm of previously independent plasma and other cells. The student who is interested in the histology of

the lesions of Johne's disease will find in the article by M'Faydean (1918) a number of photomicrographs illustrating the microscopic picture of lesions in different stages of development.

The invasion of other tissues than the wall of the intestine and the mesenteric lymph glands is apparently a rare exception. F. W. Twort and Ingram (1913) found the bronchial glands involved in one naturally affected cow. Bronchial glands have been found swollen and pure cultures of Johne's bacillus have been obtained therefrom in experimental animals. In many instances these animals have received massive doses of the organism, and it is therefore somewhat doubtful whether the changes found in such

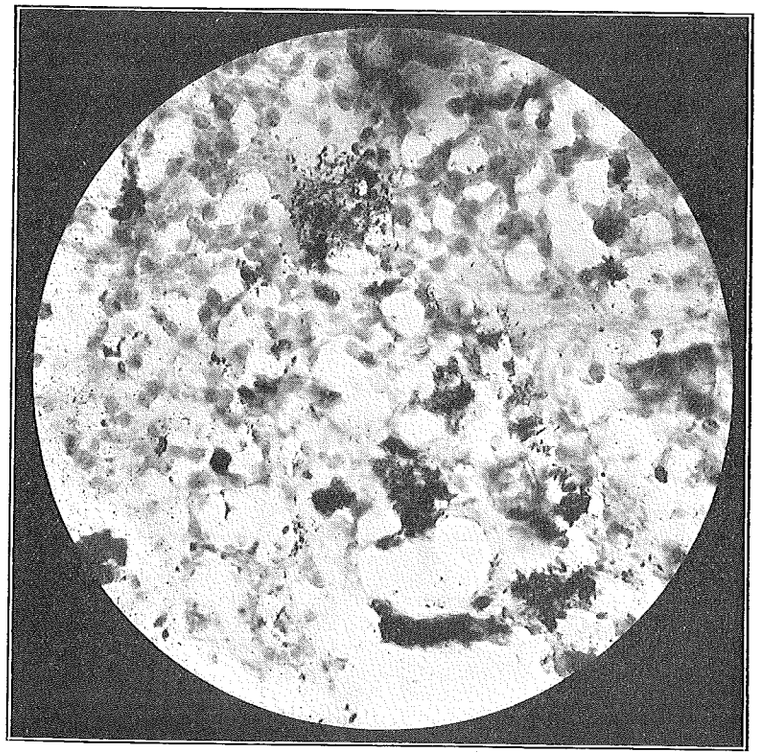

FIG. 4.—A PHOTOMICROGRAPH OF A SECTION OF A DISEASED LYMPH GLAND

The dark areas consist of masses of Johne's bacillus.

experimental animals can be considered as typical of the naturally occurring disease.

It seems that the Johne's bacillus is less toxic to the tissues in which it is growing than is the tubercle bacillus. On the other hand, the cells apparently have little restraining action on the growth and dissemination of the bacilli.

The organism is a very perfect parasite in that it does not so intrude upon the physiological processes of the animal as to cause rapid decline and death. In many instances the animal is reduced to a low level of nutrition which, however, can be maintained for months and years. Observations regarding this have been recorded on page 7.

Fatal Nature of Disease

There is no evidence to show that an infection is ever overcome. As has been stated, the period between infection and the appearance of symptoms may be many months or years in length. Symptoms may be noted for a period, which may be followed by an interval in which none is evident, and in which the animals gain flesh rapidly. Such has led to reports concerning the value of certain drugs. Sooner or later, however, the parasite begins its onward march, with the battle always ending in its favor, unless some other agency causes death before the parasite has a chance to finish its work. Apparently the same condition prevails in this disease as in tuberculosis; namely, that infection is never overcome by the bovine animal.

Elimination of the Organism

As has been stated in the discussion of the pathology of Johne's disease the necrosis and caseation so evident in tuberculosis are absent. There is, then, no opportunity for the wide-spread distribution of the organism in the animal through the flooding of the blood stream from an abscess as

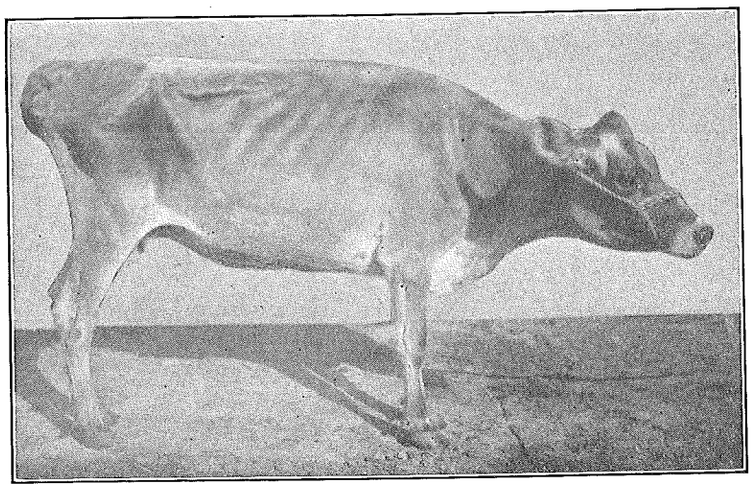

FIG. 5.—A CASE OF JOHNE'S DISEASE

The extreme emaciation is a characteristic symptom of Johne's disease. This animal was a member of a herd of 200 animals, 10 per cent of which were lost in eighteen months from Johne's disease.

occurs in tuberculosis, nor an opportunity for the organism to be eliminated in large quantities as again is true with tuberculosis.

Apparently the organism is eliminated only with the material coming from the lesions of the intestinal wall. There is constant desquamation of the epithelium and probably from the areas in which the organism is growing the desquamation would be more rapid than is normally the case. This would imply that the shedding of the organism would be a steady process rather than an intermittent discharge as is common in tuberculosis. This would also imply that the feces would be the only carrier of the Johne's bacillus.

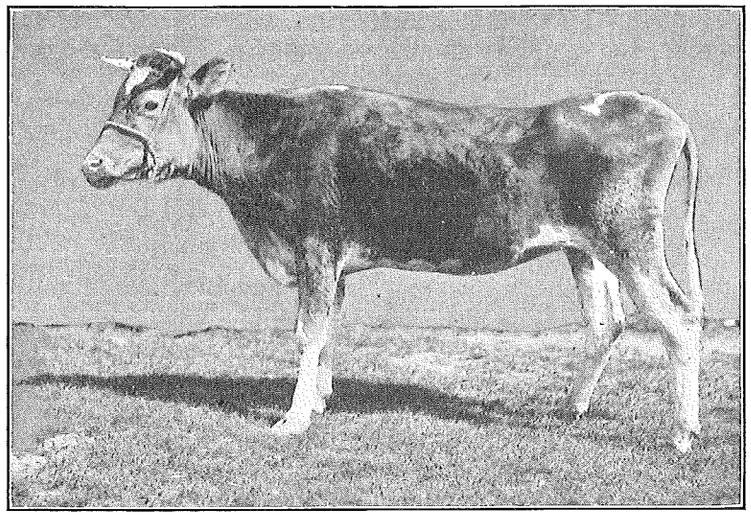

FIG. 6.—A CASE OF JOHNE'S DISEASE

This animal remained in the condition shown for two and one-half years after the picture was taken, when death resulted from the disease. Positive reactions were obtained on injecting johnin at intervals during the two and one-half years the animal was under observation.

The chronic nature of the disease and the approximate commensal relation of host and parasite undoubtedly mean that the organism may be eliminated over a period of years. The constant contamination of the stable and hence constant exposure of the animals may account for the extensive spread of the disease in a herd, in spite of the low infectivity of the causal organism.

Meyer (1914) states that Meissner and Trapp (1910) and Malm (1912) have demonstrated that affected animals give off the organism in 40 to 50 per cent of cases. Meyer (1914) claims to have established that the elimination is intermittent. Such observations must be based on a microscopic examination of the feces, a procedure somewhat questionable on account of the minute amount of material which can be examined.

The growth of the organism outside the animal has been considered by

Twort and Ingram (1914) a possibility, since they obtained some growth on media containing an extract of certain seeds. The infection with organisms which have grown in other locations than in an animal is questionable. Some have thought that the disease is most prevalent in cattle pastured on low and marshy lands. The correctness of this belief may well be doubted. From the practical viewpoint it is safe to assume the diseased animal as the only source of the organism.

How the Infection is Acquired

It seems probable that infection is acquired only by way of the digestive tract. Feeding experiments have given positive results in the hands of a number of investigators.

Krautstrunk (1918) thinks infection occurs much less easily than in the case of tuberculosis. It seems from the nature of the two diseases that this is true. In tuberculosis the organism is carried out of the body by the sputum, feces, milk and urine, and at times in immense numbers. The elimination of the Johne's bacillus only in the feces must certainly have a retarding effect on the spread of the disease as compared to tuberculosis in which there is opportunity for direct passage of the bacilli from animal to animal by licking each other, and through the soiling of mangers and watering troughs with contaminated material. The body has but *one* exit for Johne's bacillus and a number for tubercle bacilli.

The Causal Organism

Smears made from the intestinal musoca from cases of Johne's disease reveal the presence of minute acid-fast organisms, which cannot be distinguished microscopically from the tubercle bacillus. The mycobacteria, to which both the Johne's bacillus and the tubercle bacillus belong, are characteried as a group by the resistance which the stained organisms show toward decolorizing agents such as acids and acid alcohol. The resistance is not so marked in saprophytic members of the group as it is in the Johne's bacillus and the tubercle bacillus. The action of decolorizing agents on the Johne's bacillus is scarcely more marked than on mammalian tubercle bacilli and much less marked than on the avian tubercle bacillus.

The beaded appearance which is often observed in the tubercle bacillus, as well as branching forms, may also be found in the Johne's bacillus. The extreme minuteness of the organism has been emphasized by M'Faydean, Sheather and Edwards (1912), who say it is the smallest known member of the acid-fast group. This will hardly serve, however, to distinguish it from other acid-fast organisms which may be encountered in the animal, and hence no positive statement can be made on the basis of microscopic findings alone, especially where the organisms are not numerous in the tissue.

Cultivation

The isolation and culturing of the Johne's bacillus is by no means an easy task. Meyer (1914) reports that cultures of the organism are easily obtained; but the experience of numerous workers has been contrary to this statement.

Isolation

Isolation is rendered difficult first by the presence of contaminating organisms, second by the extremely slow and sparse growth of the causal organism, and third (and most important) by the fastidious taste in media exhibited by the Johne's bacillus. Regarding the first difficulty, this is overcome as in the case of the isolation of the tubercle bacillus, by the use of antiformin, to which the Johne's bacillus appears very resistant.

The suspected tissue is cut in small pieces. These pieces are then submerged in 30 per cent antiformin from 10 to 30 minutes. It is advisable to start planting the pieces of tissue on suitable media after 10 minutes treatment and continue through 30 or more minutes. By so doing one is more apt to be successful. It is not necessary to wash the tissue free of antiformin although at times it may be advisable, especially where badly contaminated pieces of tissue are treated with strong antiformin. Another

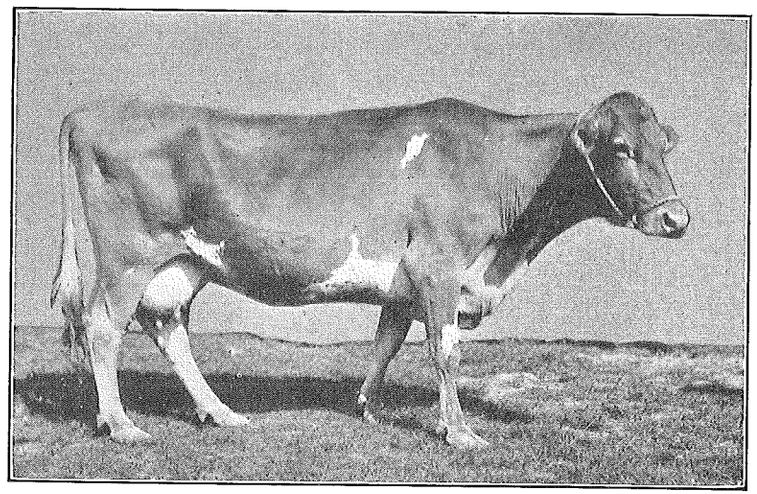

FIG. 7.—AN INFECTED ANIMAL

The animal reacted to johnin at intervals during the two years in which it was in the experimental herd. Lesions were found on slaughter.

procedure is as follows: Grind the suspected tissue in sand, add sterile physiological salt solution (20 cc). Filter through gauze to take out the coarser particles. Add pure antiformin to make 5 per cent of the total volume. After 10 minutes start seeding the media with sterile pipettes and continue through 20 to 30 minutes. Here again it is not necessary to remove the antiformin.

In our work the antiformin solution is prepared according to the directions of Cruickshank (1912).

The second difficulty is important in that tubes showing no growth after an incubation period of weeks are likely to be discarded. It has been demonstrated that on first culturing from tissue the growth may not be

perceptible on the slope for over a month, and then may be so scanty that only the closest scrutiny will reveal it. Being practically colorless and lying flat against the surface of the medium, it may appear first as a dullness on the surface which is easily overlooked by one not familiar with its characteristic appearance.

The "Essential Substance"

The difficulty of providing an acceptable medium seemed for a long time insurmountable. It was overcome by F. W. Twort (1910) who obtained the first pure culture of the organism on a medium which contained the killed cells of the human tubercle bacillus. Based on the idea that all acid-fast bacteria contain a common substance which might, if added to ordinary media, permit the growth of members not easy to cultivate, this

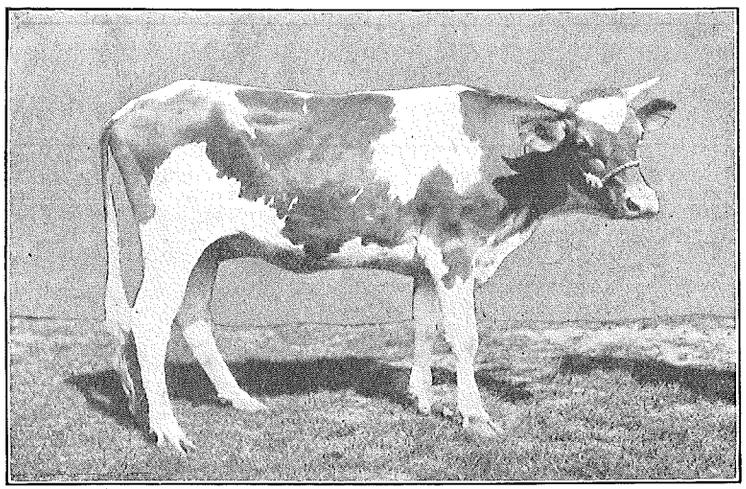

FIG. 8.—AN INFECTED ANIMAL
The animal gave frequent reactions to johnin during the two years in which she was in the experimental herd. Lesions were found on slaughter.

use of extracts and killed cells of acid-fast organisms is responsible for all the progress that has been made in the laboratory study of Johne's disease. As far as we are aware, no one has isolated the bacillus other than in media containing the cells or extracts of acid-fast bacteria.

The nature of the "essential substance" thus furnished is not known. Twort and Ingram (1914) made an attempt to determine its nature and its occurrence in other materials than the cells of acid-fast bacteria. They found small amounts of a substance permitting the growth of the Johne's bacillus in oats, flax seed, figs, the currant grape and the fungus Cantharellus aurantiacus. This "essential substance" is, however, so uniformly provided by easily cultivated acid-fast bacteria that no other sources are of practical importance.

When, as will be described later, the Johne's bacillus is used in the preparation of johnin (the specific diagnostic agent for Johne's disease) it is not possible to use tubercle bacilli as a source of the essential substance, because tuberculous cattle might react if injected with johnin made in this way. Avian tubercle bacilli have been used by a number of investigators and by us, both for the cultivation of the organism and for the preparation of johnin. Our present knowledge concerning the relation of avian tubercle bacilli to cattle makes the use of avian tubercle bacilli in the manufacture of the diagnostic agent as unsafe as the use of bovine or human tubercle bacilli or their extracts.

For this reason, and because of the more abundant and rapid growth obtainable, we have used the timothy grass bacillus, Mycobacterium phlei, which has proven satisfactory not only to us but to other students of the disease. In the early stages of our work a large variety of non-pathogenic acid-fast organisms were used in the preparation of media. The substitution of M. phlei for these has been followed by much greater success in obtaining satisfactory and consistent growth of Johne's bacillus. It is certain that many of the saprophytic acid-fasts produce a decomposition of the medium quite unlike that caused by M. phlei and the tubercle bacilli. What relation this may have to the more successful use of M. phlei in cultivating the Johne's organism is unknown.

It is not to be inferred from the foregoing statements that all difficulties in culturing are overcome when a sufficient quantity of the essential substance is supplied. The inexplicable irregularities of growth noticed in the tubercle bacillus by anyone who has worked with it over an extended period are much more marked with the Johne's bacillus. Of a series of transfers made from a single seed tube, it is not uncommon in our experience to find perhaps one-third or less positive. This positive portion may give excellent growth, while the remaining tubes are sterile. Or growth may fail to appear for a month or six weeks in any subculture, but develop uniformly and rapidly after first becoming visible. This is probably the cause of the lack of success reported by many laboratories in obtaining successful transfers from the cultures which we have supplied them, and which were known to be in good condition when they left our hands.

In liquid media seeded with a suspension of the organism it is sometimes three or four months before growth is apparent. This has been noticed by other workers who have made an extended study of the organism in culture. For example M'Faydean, Sheather and Edwards (1916) speak of the slow and inconsistent growth of a strain even after five years of cultivation on artificial media. The experience of Twort and Ingram (1912) who grew cultures on a medium devoid of the essential substance and who did not obtain visible growth until after four months will not be surprising to anyone who has tried to obtain surface growth of the Johne's bacillus on a liquid medium even where the essential substance was supplied.

We must conclude that up to the present the combination of nutrient materials best suited to support the growth of this organism has not been found. In no other way can the inconsistencies of growth be explained.

It is to be noted that on egg slopes a very heavy inoculum usually leads to the development of growth that begins as discrete colonies, while in clear broth cultures seeded with a suspension of the organism, the first indication of growth is the formation of small "pellets" in the bottom of the flask. The indications are that relatively few of the organisms introduced ever grow.

Culture Methods and Media

During the course of the work a large number of media have been tried as to their value for growing the Johne's bacillus. Acting on suggestions gleaned from the published work of others and from the experience of our colleagues at other institutions, we have added naturally sterile

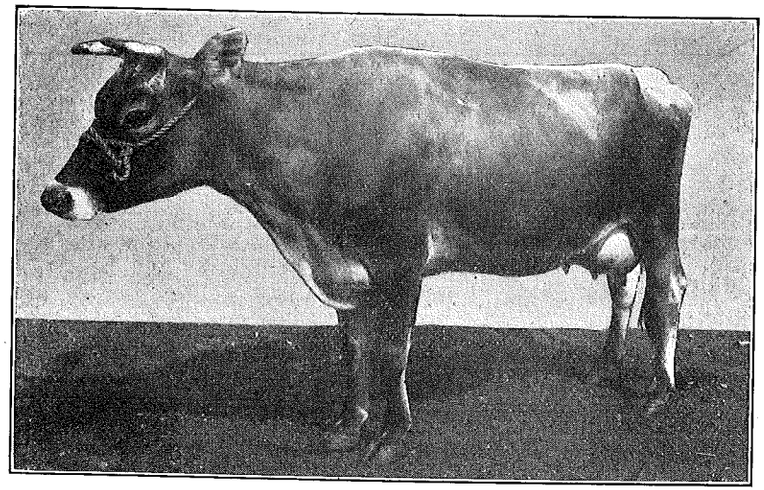

FIG. 9.—A CASE OF JOHNE'S DISEASE DETECTED BY JOHNIN
A member of a herd freed from the disease by repeated tests and removal of reactors.

serum, naturally sterile defibrinated whole blood, liver extract, etc. to infusion media, without obtaining any marked improvement. As a source of essential substance the human, bovine and avian types of the tubercle bacillus have been used, and acid-fast saprophytes of various kinds.

At present we are using only three kinds of media; these have proven the most satisfactory in our work.

The solid medium on which stock cultures are kept is made from—

Whole egg	70 per cent.
Water	24 per cent.
Glycerine	5 per cent.
Dried growth of Mycobacterium phlei	1 per cent.

Dried growth of M. phlei is generally used because one can easily pre-

pare a quantity of this material in advance and store it for future use. A weight of moist growth equivalent to 1 per cent on the dry basis is just as satisfactory in our experience. The dried growth must be finely ground and suspended in water before adding to the egg while the moist growth is easily distributed throughout the medium without grinding.

The slopes are inspissated for two hours on each of three successive days or sterilized in the autoclave, using the same precautions necessary in sterilizing serum slopes. The organism when seeded on these slopes shows growth after about three weeks at 37°. In order to prevent drying of the medium the plugs are burned off and pushed down into the tubes

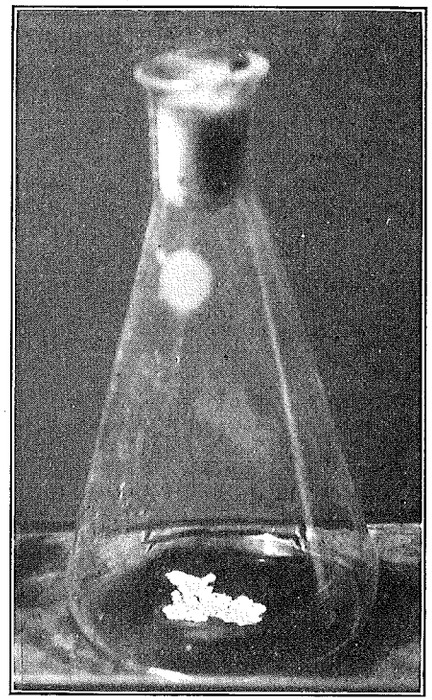

FIG. 10.—SURFACE GROWTH ON BROTH

A fair sample of the amount of surface growth obtained on infusion broth containing M. phlei in the first decade of our work.

which are then sealed with corks dipped in boiling water in order to destroy molds thereon. Growth is dull, flat and colorless, appearing as discrete colonies or occasionally in a veil-like formation following the needle strokes. On longer incubation it has a tendency to pile up. This tendency is noted in all acid-fasts, and in the Johne's bacillus is not especially marked save on an occasional tube. It is quite remarkable that cul-

tures on egg may remain in the incubator for over a year and yet give as good growth on transfer as relatively young cultures.

We have used two essentially different liquid media for the preparation of johnin. One has a beef-infusion base, the other is synthetic. The former has the following composition—

Difco peptone	1.0 per cent.
K_2HPO_4	0.5 per cent.
Glycerine	5.0 per cent.
Beef infusion	
pH	7.0-7.2 per cent.

This is combined with an equal volume of the same broth on which M. phlei has grown for about two weeks. The phlei cultures are killed by steaming for a short time and filtered through paper, the filtrate being used as described. When this medium is seeded with a suspension of the Johne' organism, growth begins in a month to six weeks as small pellets floating free near the bottom of the flask. These when examined microscopically are found to be clumps of acid-fast bacteria. The medium never becomes turbid. Surface growth may begin after a lapse of several months but will not invariably do so. If it does appear, it may be thick, wrinkled and brittle, or thin and fragile. In the former case it does not extend to cover the surface of the medium. In the latter it does cover very rapidly. The growth on liquid as on solid media is never pigmented. At most it shows a faint creamy tint.

The synthetic liquid medium has been described by Dorset for the cultivation of tubercle bacilli. With the addition of moist killed growth of M. phlei it becomes an excellent medium for the Johne's bacillus. The composition of this medium is—

K_2HPO_4	0.1 per cent
Sodium citrate	0.05 per cent
$MgSO_4$ 7 H_2O	0.1 per cent
Asparagine	0.5 per cent
Glycerine	7.0 per cent
Ferric citrate	0.006 per cent
Moist growth of M. phlei	0.5-1.0 per cent

The reaction requires no adjustment.

Subsurface growth is not of course discernible in this medium because of the sediment of M. phlei cells. Surface growth is abundant, heavy and wrinkled, climbing the walls of the flask. Its maximum is reached in three or four months, if it has been seeded with surface growth, at which time the culture resembles one of the tubercle bacillus in quantity and character of growth.

We have prepared johnin from cultures on both kinds of liquid media by evaporating the whole culture to one-tenth, diluting to the original volume with 0.5 per cent phenol solution as required for use and filtering through paper. These preparations have been repeatedly tested on animals affected with Johne's disease, 10 cc. being used as a dose, intravenously.

Identification of Cultures

Because it is not an uncommon thing for acid-fast organisms to be isolated from healthy animals, and because (as will be mentioned further on in detail) acid-fast organisms other than the Johne's bacillus have been repeatedly isolated from cases of Johne's disease, the identification of cultures is an important consideration.

The Johne's bacillus is not easily identified. It does not produce any characteristic change in laboratory animals. The only animals known to be affected by it are cattle and sheep, and these cannot be commonly em-

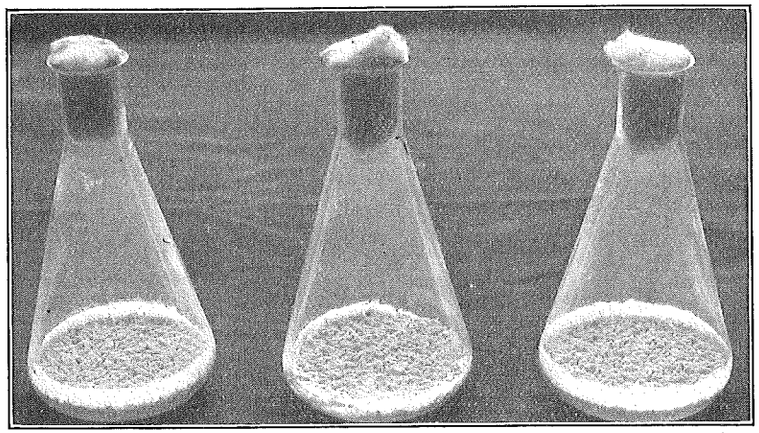

FIG. 11.—JOHNE'S BACILLUS ON THE SYNTHETIC MEDIUM
Growth after three months' incubation.

ployed to identify cultures. One must, therefore, rely on the cultural characters in large part. An acid-fast organism, isolated from what are regarded as characteristic lesions, which grows very slowly and only on media containing extracts of acid-fast bacteria, is, according to our present knowledge, likely to be the Johne's bacillus. The lack of pigmentation in old cultures is also, we believe, an important point in differentiating Johne's bacillus from other acid-fast bacilli. No color has been noted in any of the strains of Johne's bacilli in our collection, even when the growth was profuse and months old. The avian tubercle growth develops a salmon pink color and a strain of acid-fast bacillus which was sent us as having been isolated from lesions of Johne's disease, a yellow color.

In our stocks at the present time we have six strains of what we believe to be the true Johne's bacillus. Two of these were isolated by Dr. W. A. Hagan at Cornell, three were isolated in this laboratory and one was sent us from London by Dr. F. W. Twort. We have identified these cultures by their cultural and growth characters, which are identical, and by the fact that animals affected with Johne's disease react when injected with diagnostic agents prepared from them.

Aberrant Strains

In the attempt to culture the Johne's bacillus from lesions the isolation has frequently been reported of acid-fasts which, judging by their cultural characteristics, were probably not the organism sought. Some workers have believed them to be the Johne's bacillus; others have been convinced that they had the avian tubercle bacillus. These isolations of aberrant strains are so frequent that they cannot be disregarded in the consideration of the laboratory study of the disease. The infection with acid-fast organisms may be a secondary invasion to the original infection with Johne's bacillus; on the other hand there seems reason to believe that if it is connected in any way with Johne's disease the presence of other acid-fasts may be considered primary and the Johne's invasion secondary. A number of tissues of healthy animals cultured by us have yielded acid-fast organisms of which up to the present no detailed study has been made. We are certain only that they are not identical with the Johne's bacillus.

In two cases the lesions from cows which showed typical symptoms and which had given positive reaction to johnin were cultured for the causal organism, and rapidly growing acid-fast cultures were obtained. These cultures while isolated at different times from different herds have certain distinguishing characteristics in common. Some subcultures from a seed tube show sparse, colorless growth and some from the same tube show very heavy, pigmented growth. Whether the sparse or heavy growth is used as seed, the subcultures show the same lack of uniformity. The organisms produced death in sheep on intravenous inoculation. They were also fatal to rabbits and hens but not to guinea pigs. They seem to be avian tubercle bacilli or closely related to them. No data are available as to the presence of tuberculosis on the farms from which the animals came.

We have in our collection another culture of an acid-fast organism, said to have been obtained from typical lesions of Johne's disease, which was sent to us by a commercial laboratory. No animal tests have been made with it, but the fact that it is not the Johne's bacillus seems evident because of its rapid growth on plain glycerine peptone broth. It develops a yellow pigment in old cultures.

A number of instances might be cited to illustrate the isolation of aberrant strains. Thus, Albeini's (1910) culture gave visible growth on Nährstoffheyden; Dammann and Stedefeder (1910) obtained from the intestines of a calf an easily cultivable acid-fast organism; Melvius' (1909-10) organism gave profuse growth on media now recognized as unsuitable for the Johne's bacillus. The assertion of Mellon and MacGinnis (1913) that their culture grew at 25°C. indicates either mixed cultures of acid-fasts or some organism other than Johne's bacillus.

A mixed culture of the Johne's bacillus and of tubercle bacilli was obtained by M'Faydean and Sheather (1916).

We consider particularly significant, however, those cases where the bacillus isolated appears to be similar or identical to the avian tubercle bacillus, and cannot agree with Meyer (1913) that such findings are purely accidental. The fact that avian tuberculin has in years gone by been used as a diagnostic agent for Johne's disease seems to signify a relationship.

More recently Plum (1912) has shown that animals infected with avian tubercle bacilli may react to johnin. We do not know that avian tubercle bacilli will produce symptoms and lesions which may be mistaken for Johne's disease. It may be that there was a mixed infection with Johne's organism and the avian tubercle bacillus or some other acid-fast bacillus.

Meyer (1913) reports that Koch and Rabinowitsch (1907) studied a culture isolated by Stuurman and asserted it to be an avian tubercle bacillus. He states that Maltam and Miessner isolated bacilli which corresponded in every respect with avian tubercle bacilli.

To us the frequency of isolation of what are apparently avian tubercle bacilli from supposed lesions of Johne's disease deserves consideration.

Pathogenicity

Among the most helpful procedures in the study of a disease and its causal organism are transmission experiments, not only with the natural host animal, or animals, but also with laboratory experimental animals. When the possibility of using small experimental animals is excluded, the study of the disease is likely to be slow and expensive. Such is the case with Johne's disease.

The following is a review of the various inoculation experiments of previous investigators.

Johne (1895) used material in which he had discovered the organism to inoculate guinea pigs; his results were negative. Markus also obtained negative results on inoculating with infective material guinea pigs, rabbits, goats and hens. Holth (1912) inoculated rabbits and guinea pigs with pure cultures but was not successful in producing the disease.

B. Bang (1906) obtained negative results with guinea pigs and rabbits, but did produce the disease in calves by feeding the mucous membrane of an infected animal.

Miessner and Trapp (1910) inoculated and fed calves with material from cases of Johne's disease. One calf showed lesions; the other as well as the laboratory animals and hens, gave negative results after three to six months. In another series of cases, seven in number, the only positive results were with three calves inoculated intravenously, one with the mesenteric fluid and two with the intestinal mucosa of a cow suffering with Johne's disease.

Meyer (1913) was able to produce the disease in calves by intravenous inoculation with mesenteric lymph nodes from naturally occurring cases. The period during which the animals were allowed to live after inoculation ranged from 12 to 16 months. Feeding trials with material of the same origin were negative in the two cases completed at time of publication. Two others were still under observation.

Some of the animals used by Meyer were several months old. One was approximately one year old. Meyer emphasizes that others had used calves in their transmission experiments. He would explain their positive results on the grounds that young animals had been used. His negative feeding

trials with older animals indicate to him the increased resistance to infection of older animals.

From these facts he concluded that natural infection occurs early in life, and that in any effort to combat the spread of the disease, attention should be directed especially to the calves which should be removed from contaminated surroundings immediately after birth.

Twort and Ingram (1913) report approximately 50 per cent successful inoculations on calves with pure cultures. The animals with one exception were killed 6 to 12 months after inoculation. One killed in two months showed no evidence of the disease.

The most extensive inoculation experiments with laboratory animals have been made by C. C. Twort and his associates. In 1912 in his study of the complement-fixation and agglutination tests for the diagnosis of Johne's disease, he found that rabbits and fowls respond to injections by producing antibodies, but do not acquire the disease. Together with Craig (1913) he reported that it was impossible to produce Johne's disease by injection in experimental animals. In 1914 he reported on a much more extensive series of trials, the results of which are summarized in the following table:

11 rabbits inoculated intravenously	5	positive
8 rabbits inoculated intraperitoneally	0	"
4 rabbits fed	0	"
28 mice inoculated intraperitoneally	5	"
12 mice fed	1	"
12 rats inoculated intraperitoneally	3	"
6 guinea pigs inoculated intraperitoneally	0	"
81	14	"

The culture used in the work was one which had been acclimatized to ordinary glycerine beef broth, not containing the essential substance. The culture appeared to be more pathogenic than the original culture grown on media containing M. phlei.

The doses were massive and in some cases were repeated two or four days following the first injection. The results in such cases can hardly be considered as especially significant regarding the disease. In fact, it was stated that spontaneous recovery will probably take place in the rabbit if it is protected from reinfection.

In the case of the rabbits inoculated intravenously six showed no evidence of disease, and five evidence. The infection was limited to the sacculus rotundus of the ileum and about a foot of the ileum itself. The organisms were found in the swollen villi, in the abdominal and thoracic lymph glands and more rarely in those of the axilla, and also in the liver and spleen.

The intraperitoneal injection of rabbits produced no intestinal lesions. Cultures were made after 157 days from the mesenteric and thoracic lymph glands with positive results.

The feeding of rabbits was likewise ineffective in producing lesions. A few colonies developed on media inoculated from the mesenteric glands nine months after feeding.

Only a small part, five out of 28, of the mice injected intraperitoneally developed the disease. These, however, showed it in a very extensive form, the entire intestine from stomach to rectum, being involved.

Results were wholly negative with six guinea pigs.

Twelve rats were inoculated intraperitoneally. A few acid-fast bacilli were found in the ileum in two of the animals and in the duodenum of another.

The thought which the results of Twort produce is that the Johne's bacillus is not truly pathogenic for any of the laboratory animals. Massive and repeated doses may in some cases produce changes reminding one of those found in the naturally infected bovine.

The results of culturing animal tissue for acid-fast bacilli and concluding that organisms obtained in the absence of lesions characteristic of the disease are identical with those introduced seem to be somewhat unsafe in view of the almost total ignorance which exists concerning the occurrence of such acid-fast bacilli in the normal animal.

The most illuminating trial with experimental animals involved but a single animal, a rabbit, inoculated by O. Bang and Andersen (1914) subcutaneously with material from a goat. The animal appeared in perfect health 18 months after injection. A post-mortem examination revealed a thickened intestinal wall, in which were found masses of acid-fast bacilli, which behaved like Johne's bacillus.

This experiment may carry an important message to those studying Johne's disease, namely that periods of time which suffice with tuberculosis, etc., are not sufficient when such a slowly developing disease as the one in question is concerned. It may be that much longer periods of observation would be fruitful.

We have injected sheep both intravenously and subcutaneously. A thermal reaction was noted within a few hours and persisted for 10 days. No other symptoms have been noted to the present time, one year after injection. The infection seems established since all the animals have repeatedly given positive reactions to johnin.

Diagnosis on the Living Animal

Microscopic Examination of the Mucous Membrane

The limitations of the lesions to the intestinal wall and the abundance, in many cases at least, of acid-fast bacilli therein led to the method of diagnosis by introducing the arm into the rectum, pinching off a bit of the mucous membrane and examining it microscopically for acid-fast bacilli. It is at once evident that such a method of diagnosis can not give an unequivocal answer as to the presence of the disease. Only a tiny area of the intestinal wall can be examined and hence absence of acid-fasts therein is no evidence of absence of the disease. Even the significance of acid-fast bacilli may be questioned if the animal shows no other evidence of the disease. The method may have been of some value earlier, but it is certain that with it nothing of importance can be done in freeing a herd from the disease.

Avian Tuberculin as a Diagnostic Agent

In the early study of Johne's disease the causal organism was thought, by some, to be the avian tubercle bacillus. This supposed relation, together with the difficulty of cultivating the Johne's bacillus, probably led to the use of avian tuberculin as a diagnostic agent. Much work was done both in an experimental way and in the field by a number of workers. Edwards (1926) believes that avian tuberculin can be used effectively when the herds in question have been shown to be free of bovine tuberculosis by testing with mammalian tuberculin.

O. Bang (1914) was not successful in freeing herds from Johne's disease by testing with avian tuberculin and by the removal of reactors. The herds were large and the disease was prevalent therein, from 9 to 45 per cent of the animals over two years old reacting. He believed the lack of success was due to non-reaction of some animals not showing clinical symptoms and stated that this could be proven in some cases.

Schalk (1926) has shown that on cohabiting under experimental conditions with tuberculous fowls, cattle soon become sensitized to avian tuberculin. He has also shown that animals shipped to the stock yards may react to avian tuberculin, the supposition being that such animals have become sensitized on the farms by association with tuberculous fowls.

Plum (1925) in his studies in Denmark has also shown the invasion of cattle under natural conditions by the avian tubercle bacillus and the reaction of such cattle to both subcutaneous and intradermal injections of avian tuberculin.

The prevalence of avian tuberculosis in many of the important dairy districts, and the association of fowls and cattle to some degree at least would seem to make the use of avian tuberculin questionable. According to Plum's observation (1925) the avian tubercle bacillus might pass from animal to animal in the discharges after abortion due to it.

It may be that future work will show that avian tuberculosis is of much greater economic importance in cattle than has been thought, and that the future problem will be the freeing of the herds from all acid-fast infections, rather than from any one. Whatever group relationships the human, the bovine, the avian tubercle bacilli and the bacilli of Johne may have will then be of little practical importance, since a reaction to the diagnostic agent made with any one would indicate an infection with some member of the group. Until the struggle against acid-fast infection in cattle has reached this development it is doubtful whether avian tuberculin can be successfully used to combat Johne's disease.

The data collected concerning the prevalence of Johne's disease by testing with avian tuberculin are made questionable by our recently acquired knowledge of the sensitization of cattle to this product through invasion by the avian tubercle bacillus.

Johnin as a Diagnostic Agent

The use of johnin as a name for the product made in a manner similar to tuberculin was suggested by M'Faydean (1914). The term "paratuberculin" is still used by some, Plum (1925).

At first the Johne's bacillus was grown in a medium containing extracts of one of the types of tubercle bacilli. Objections were made regarding the use of such a product on other than known tuberculosis-free animals, the basis of the objection being that the content in tuberculin might be sufficient to cause a reaction in tuberculous animals. It was thought that avian tubercle bacilli could be used without introducing disturbing factors. As has been described, this hope may not have had a safe foundation. Later M. phlei was employed to supply the essential substance. So far as we are aware, the extracts of this organism will not produce a reaction in an animal infected with any one of the types of tubercle bacilli or with Johne's bacilli.

The meager growth of Johne's bacillus which was earlier obtained both by ourselves and others led to the inclusion of the dead cells thereof in the diagnostic agent. Some objected to the use of such a preparation on the ground that, because of meager growth of the organism, the potency might be so low as to give uncertain results in the field, and again on the ground that the dead cells might sensitize healthy animals to subsequent applications of johnin. The use of serum in the medium might also introduce the danger of inducing anaphylactic shock on retest of animals. This proved a disturbing factor in our earlier work, and possibly caused one fatality.

It is now possible to grow Johne's bacillus in a simple medium, containing no serum, in which the essential substance is supplied by M. phlei which, as has been stated, introduces no known disturbing factor in the use of johnin. Growth is quite consistent and in some cases as profuse as is that of the tubercle bacillus. There seems to be no reason why johnin should not be used as successfully as tuberculin. Only wide-spread use in the field will reveal its weakness or its strength as a weapon against Johne's disease. Johnin, made with one of the cultures isolated by us, has recently (September, 1927) been placed on the market by a commercial firm.

Not long ago it was shown that the cells of the tubercle bacilli are most completely freed from the active principle of tuberculin by extraction at low temperatures, Cumming, (1926). Practically all tuberculin is made by the original Koch method, namely extraction of the cells at boiling temperature or above. It seems as though the success of the Koch method may be due to the fact that during the prolonged incubation there is opportunity for the maximum growth to be obtained, and for many of the cells to have died and to have autolysed to some extent. In other words, the Koch procedure may incidentally involve the extraction at low temperatures before the application of heat in the later stages of manufacture.

The degree of autolysis varies directly with profuseness of growth. For example, the tubercle bacillus will grow very slowly and sparsely in a medium containing casein as the only source of nitrogen. In such a medium the cells remain viable several times as long as on the usual medium which yields profusely. In the sparsely growing cultures of Johne's bacilli autolysis is slight, and hence the active principle of the johnin may be difficult to extract if high temperatures alone are used. In our own work we have used methods comparable to those used in preparing tuberculin.

Vallée and Rinjard (1926) tried a number of methods of extracting the cells and concluded that an extraction at ice box temperature with distilled water gave the most potent product. They have therefore adopted this plan for their work, both experimental and field. Dr. W. A. Hagan of the New York State Veterinary College in a private communication describes his method of extracting first at ice box temperature and later in the autoclave. The process which will yield the most potent johnin is still questionable. It seems, however, that the methods of Vallée and Hagan may prove the more effective in obtaining a potent product than the process which involves heating the cultures immediately after their incubation.

Injection of Johnin

Our field experience has been wholly with intravenous injections. Vallée and Rinjard (1925) claim good results with intradermal injections, and Plum (1925) has used both subcutaneous and intradermal injections in his study of the infection of cattle with the avian tubercle bacillus. The advantages of the intradermal and the subcutaneous application over the intravenous are evident, and it seems probable both will be found practical.

In the intravenous injection the head of the animal to be injected is secured with a halter as high up as possible and to one side. A rope is placed around the neck just in front of the shoulders and drawn tight enough to distend the jugular vein into which the johnin is introduced. The intradermal and subcutaneous injections are made as in the tuberculin test. There are little data on which to base any judgment as to dosage. In our earlier work when the growth of the cultures was meager, 10-15 cc. were used, corresponding to an equal volume of the original culture medium. With more profuse cultures 5 cc. have been used. Plum (1915) used 10 cc. of 15 per cent solution, the base material being apparently the original cultures concentrated to 1-10 their volume as is common in making tuberculin. Much smaller amounts must be used in the intradermal test. It should be recognized here as with many other phases of the johnin test that the matter is still on an experimental basis. Extended field experience is necessary to determine the best procedure.

Reaction to Johnin

The conditions under which the test is applied should be comparable to those observed in making the tuberculin test. Since the rise in temperature in a positive reaction may not, on the average, be so marked as in the tuberculin test, it is important to avoid conditions that shall affect the temperature of the animals. It seems especially important to avoid the injection of those which show an abnormally high temperature (103°F.). If such temperatures are noted in any considerable portion of the herd on taking the preliminary temperatures, it seems wise to defer the test since the condition responsible for the fever in some may, at some time during the test, cause a fever in animals which at the beginning showed a normal temperature. O. Bang (1914) believes that when the preliminary temperature does not exceed 102.2°F. that a rise to 103.6°F. may be considered as a positive response.

It seems clear that the thermal reaction will appear more quickly following the intravenous injection than following the subcutaneous. However, so little is known regarding the matter that it seems wise to begin the post injection temperatures within one hour after injection, either intravenous or subcutaneous, and to continue to the 18th hour at least.

Table IV presents a summary of our observations concerning the hour after intravenous injection at which the maximum temperature was reached.

Table IV.—Hour at Which Maximum Temperature Was Reached After Injection of Johnin into Diseased Animals

Hour after injection	Number reacted	Per cent	Hour after injection	Number reacted	Per cent
1	none	none	7	18	22.7
2	none	none	8	4	5.5
3	8	10.12	9	1	1.16
4	5	6.32	10	3	3.70
5	34	43.	11	1	1.16
6	5	6.32	12	none	none

It will be seen from the data that approximately 80 per cent of the highest temperatures fell between the fifth and eighth hours. The increase in temperature following the injection of johnin is presented in Table V.

Table V.—Percentage Distribution of Reacting Animals According to Maximum Temperature Attained on Injection of Johnin

Maximum temperature	Number	Per cent
103-104° F.	26	31.3
104-105° F.	35	42.1
105-106° F.	11	13.2
106-107° F.	8	9.6
107-108° F.	3	3.6

These data were obtained with johnin of a low degree of potency compared to that now available if the amount of cells per unit volume of the medium is a criterion of potency. A more potent johnin might have some effect in increasing the thermal rise. Ernest (1927), employing a johnin which should have been more potent than that used in most of our work, with 5 cc. injected intravenously obtained the majority of the maximum temperatures within three to six hours following injection. The temperature rise was approximately the same as in a reaction to tuberculin both in extent and duration.

Among the objections raised to johnin by O. Bang (1914) was the lack of knowledge concerning its effect on healthy animals. We believe our results have shown that this objection is without foundation.

There are manifestations of sensitization following the administration of johnin other than a rise in temperature. The majority of infected cattle exhibit a roughened hair coat from 30 minutes to 4 hours following the

intravenous injection. The condition is more noticeable in some cattle than in others. We have never seen a roughened hair coat that was not accompanied by a thermal reaction, and that did not correspond approximately in time of appearance with the initial rise in temperature.

At any time between 4 and 24 hours following the administration of johnin, approximately 25 per cent of infected cattle exhibit a marked softening of the feces. At times a severe diarrhea is noted, the bowel discharges being thin and watery, and rarely streaked with blood. A foul odor is sometimes to be noted.

Sometimes an uneasiness accompanied by muscular tremors and a more or less dyspnea may be seen. These symptoms usually appear 15 to 30 minutes after injection and persist for from one to two hours.

In three cases out of approximately 1,000 cattle tested severe constitutional reactions were noted. The first case was an adult cow that had shown clinical symptoms of Johne's disease for about two months. About one minute after the intravenous injection of johnin she fell prostrate and remained in an unconscious condition for several minutes; temperature was 102.6. The highest preinjection temperature was 101.2. The pulse was not perceptible. In about one and one-half hours recovery had apparently taken place. She reacted at this time. Post-mortem examination revealed infection with Johne's bacillus.

The second subject was a three-year old heifer that had been tested six months before, but did not react. About one and one-half hours after injection this heifer fell prostrate; dyspnea was marked; the temperature was 101.2. The pulse was not perceptible. In about one and one-half hours recovery had apparently taken place. She reacted at this time. Post-mortem examination revealed infection with Johne's bacillus.

The third case was that of a six months old heifer. About 15 minutes after injection she was markedly dyspnic with a pronounced flank breathing, the tongue protruded and the temperature had risen from 101.8 to 102.6. In about 30 minutes she was dead. Post-mortem examination revealed marked congestion in about 12 inches of the ileum situated about two feet from the ileocecal juncture. Acid-fasts were demonstrated microscopically.

Ernest (1927) noted constitutional reactions in a number of reacting animals.

Confirmation of the Test

In a part of the cattle reacting to the johnin test, we have had opportunity for confirmation of the results by a retest. In one herd, consisting of 18 animals ranging in age from two to 12 years, five reactors were found. These reactions were all definite; the lowest post-injection maximum temperature was 104.2. This herd was kept intact and retested one year later with the result that the same animals again gave definite reactions, and in addition a four-year old cow that had failed to react the year before. In the meantime, no clinical cases had developed. The only irregularity in connection with this herd was the fact that one cow gave a suspicious reaction to the first test and failed to react on retest. Five

cows in another herd reacted to the johnin test. On retest five months later two gave definite reactions and one a suspicious reaction.

In a portion of the reacting animals, opportunity for post-mortem examination has been presented. No lesions outside the digestive tract and adjacent lymph glands which could be attributed to this disease have ever been noted. Our observations are based on 24 post-mortem examinations of cattle reacting to the johnin test. Four of this number revealed no macroscopic lesions of disease; three, a slight enlargement and reddening of the ileocecal valve with no visible intestinal lesions; four, a marked enlargement and reddening of the ileocecal valve with no visible intestinal involvement; two, a marked intestinal thickening with no visible changes in the valve; and seven, marked involvement of both intestine and valve. Four showed a slight involvement of both valve and intestine.

The appearance of a piece of infected gut in Johne's disease is no indication as to the number of acid-fast organisms which can be demonstrated microscopically. They may be numerous in material showing slight lesions and difficult to demonstrate in a markedly thickened gut.

The specific organisms can usually be demonstrated by histologic section and many times also by smears. In specimens in which we have been unable to demonstrate acid-fasts readily by direct examination we have substituted a method of concentration by means of antiformin. The mode of procedure has been as follows:

A small piece, not more than ¼ inch square, of the suspected intestine is placed in full strength formaldehyde for from one to two hours, depending on the size of the tissue. It is then removed and placed in the incubator or drying chamber until thoroughly hard and dry. The tissue is then ground to a fine powder in a mortar and this powder treated for two hours with a 25 per cent antiformin solution. This is diluted with an equal volume of distilled water and centrifuged. The supernatant liquid is decanted, the tube filled with distilled water and again centrifuged. The sediment is examined for acid-fasts. This method has given good results.

Acid-fast organisms have been found in the tissues of 36 of the 37 reacting animals examined.

Intradermal Testing with Johnin

Our experience with the intradermal test has been so limited that nothing need be said regarding it. Vallée and Rinjard (1926) disregard any swelling which does not persist beyond the 24th hour following injection, since the swellings which appear soon after the injection and which rapidly disappear are not to be considered as due to the active principle of the johnin, but to some non-specific substance therein. They say that with animals artificially sensitized by subcutaneous inoculation, the reaction both with the intradermal and ophthalmic test are comparable in all respects to those in the like tuberculin tests. However, with the same johnin, Vallée and Rinjard obtained less satisfactory results on 30 animals naturally infected. The authors do not state the grounds on which they judged the animals to be infected. It would seem necessary to gain our knowledge concerning the intradermal use of johnin on animals shown to be infected by the intravenous test, rather than on artificially infected animals.

Other Methods of Diagnosis

Efforts were made by C. C. Twort (1912) to employ the complement-fixation test and also the agglutination test in the diagnosis of Johne's disease, but with little if any success. These tests have not, as far as we are aware, been successfully used in the diagnosis of any of the diseases caused by the different acid-fast bacteria. Group reactions are likely to

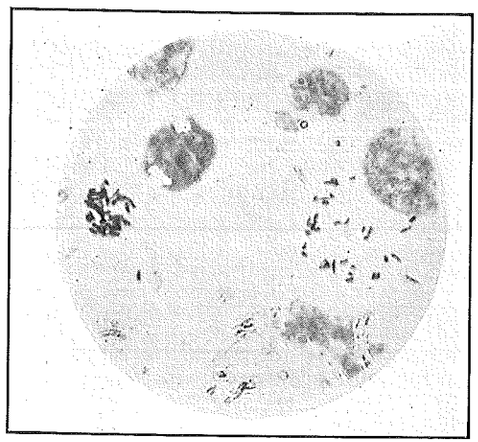

FIG. 12—JOHNE'S BACILLI IN A MESENTERIC LYMPH GLAND

The smear was stained with carbol-fuchsin, decolorized with one per cent hydrochloric acid in alcohol, and counterstained with methylene blue. Magnification 1,250 diameters. Clumps, individual bacilli and tissue cells shown.

prove a disturbing factor. C. C. Twort (1912) states that for the production of agglutinins and amboceptors it makes no difference whether one uses Johne's bacillus, M. phlei or the avian tubercle bacillus, and that the specificity within the acid-fast group is very limited.

Lewis and Aronson (1923) in their work on leprosy, and Verge (1923) in his work on the complement-fixation test for the diagnosis of tuberculosis noted that the specific antigen was no more efficient than other antigens made with other members of the acid-fast group. The group relations enter into all the tests used for the diagnosis of acid-fast infections probably to a greater extent than has been recognized in the past. M'Faydean, Sheather and Edwards (1916) state that johnin may produce reactions in tuberculous animals. Plum (1925) has shown that reactions may be caused by johnin in animals sensitized by avian tubercle bacilli. Avian tuberculin causes reactions in some animals infected with Johne' bacillus, while mammalian tuberculin does not.

Results of Herd Tests

The original purpose of this project was to detemine the possibility of eliminating the diseased from herds, through use of the methods shown to

be effective in eradicating tuberculosis. It thus involved the isolation of the causal organism, its continued cultivation, the preparation of the diagnostic agent and its use in the field. It was recognized that the slow progress of the disease in the individual animal would undoubtedly make the task of freeing a herd from the disease a long one. Practically nothing was known concerning the length of the period of incubation, nor was anything known concerning the stages of the disease in which an animal may not react to the test.

The herd on which the most work has been done was known to have been infected for 14 years at the time the work was begun. During this period 20 animals had been lost because of the disease. The average number of animals in the herd was 45. Table VI presents the results obtained in the various tests made on this herd.

No tests of the herd have been made since 1923. It is believed that the herd is free from the disease, since no clinical cases have been noted in the 4½ years which have elapsed since the last test.

Table VI.—Results of Test on Herd I

Date	Reactors
June, 1917	5
November, 1917	4
February, 1919	6
June, 1919	3
November, 1919	4
June, 1920	4
December, 1920	2
June, 1921	0
July, 1923	1

The owner of this herd felt very hopeless in his struggle against this disease because of the fact that there was no way of ascertaining which were the infected animals until clinical symptoms appeared. It is probably true that long before these symptoms are to be noted the organisms are being given off by the affected animal. It was hoped that the test would enable the affected animal to be recognized before she became a source of danger to the other members of the herd. It was this hope that led the owner to continue the use of the test in his herd.

The specific organism has been found in the tissues of all but one of the 28 animals examined. The animal reacting in 1923 was not examined for acid-fast bacilli. In the test made in June, 1919, one adult animal gave a suspicious reaction. At the following test in November, 1919, there was no indication of a reaction to the johnin. The animal did, however, give a clear cut reaction in June, 1920, and was removed from the herd.

Another herd has been tested once yearly for three years. One reactor was found in the first test. No animals have reacted during the 2nd and 3rd tests. One clinical case developed in this herd. The infection was demonstrated by post-mortem examination in both the clinical case and the animal reacting to the test. The work on the other herds which we have under observation has not been extended enough to warrant its inclusion here.

Spread of the Disease in a Herd

Some observations have been made on the rapidity with which the disease spreads in a herd. In 1910, three animals were introduced into a herd. Two of these showed clinical symptoms in 1912 and were sold. The other showed symptoms of the disease in 1913. The disease was undoubtedly introduced into this herd by these animals. During the period 1913-21, 15 animals had been removed from the herd because of this disease.

Another herd became infected in 1905 through the introduction of an animal that apparently at that time was showing clinical symptoms. Two years later another animal was disposed of because of marked symptoms of the disease. In 1909, six animals were removed from the herd because they were in the last stages of the disease. No other clinical cases developed until May, 1916, when 2 animals were removed. Between June, 1918, and January 1, 1919, five clinical cases developed, and the animals were disposed of. In January, 1920, the herd was first tested. Seven animals reacted and were removed. In December, 1920, the test detected 11 reacting animals; 6 of these were removed from the herd and the remaining 5 were retested in May, 1921. Two gave positive reactions and one a suspicious reaction. No rise in temperature was noted in the other two.

It is evident from these observations that the disease is one which at times may spread rapidly in a herd, and at other times it may spread exceedingly slowly.

Treatment of the Disease

As has been stated, one of the most pronounced symptoms of the disease is an intermittant diarrhea. Since astringents have been commonly used to overcome diarrhea, many veterinarians have thought they were indicated in the treatment of Johne's disease. M'Faydean (1914) reported on a case which he then considered had been successfully treated. One ounce of the following solution

ferrous sulphate	5 ounces
dilute sulphuric acid	5 ounces
water	1 pint

was administered daily. The animal soon showed a marked increase in weight and exhibited a favorable appearance in comparison with that presented at the time the treatment was begun. Later M'Faydean, Sheather and Edwards (1915) reported that the improvement noted in the previous paper had not proved to be permanent. The animal began to fail rapidly and the former treatment now had no effect in stopping the diarrhea.

Reports were also made on the similar treatment of eleven additional animals. It was concluded that whatever effect was shown was due to the ferrous sulphate, the general conclusion from the work being that while the treatment seemed to exert a favorable effect, the improved condition was only temporary. The authors suggested that such a treatment might be of some assistance in prolonging the life of valuable pregnant animals. Edwards has also suggested that such treatment might be used to bring an animal into such condition that it could be sold for slaughter.

Manuel (1914) also speaks of the successful treatment of a case of the disease with sulphuric acid and copper sulphate. It is not clear from this report that the case in question was one of Johne's disease.

In view of the fact that very favorable reports have been made concerning the effect of chaulmoogra oil on leprosy, and because of the report concerning its inhibiting effect in vitro on the acid-fast bacteria, especially the tubercle bacillus, Walker and Sweeney (1926), Lindenberg and Pestana (1926), it seemed wise to try this treatment on a clinical case of Johne's disease.

Originally in the treatment of leprosy the oil was administered by mouth. Due to its disagreeable taste prolonged treatment was usually not possible. More recently injections of the esters of the fatty acids of the oil have been employed.

In our own work 5 cc. of the oil was mixed with the grain fed on each day. At first the animal rejected the food, but later became accustomed to the taste of the oil. The treatment was continued for 400 days. Improvement in the appearance of the animal followed the treatment. We are not at all certain from the trials on this one case and from our experience with the disease that we were not working with an animal during a period when the progress of the disease was naturally retarded, and that the same results would not have been obtained without the oil. That this cow continued to harbor the specific organism is evidenced by the fact that she reacted to johnin more than a year after the treatment with chaulmoogra oil was discontinued. Only extended trials on clinical cases can show the effect of the treatment. The cost of the oil is not so great as to preclude its use in the case of valuable animals.

We believe that there is little reason from observations made to the present time to conclude that any treatment is effective in retarding to any extent the progress of the disease which may be marked by its ebb and flow in the animal over long periods of time.

Schütze and Zilva (1927) report that the feeding of sodium chaulmoograte did not prevent the development of omental tumors that appear in rats after intraperitoneal inoculation with tubercle bacilli. The authors find the sodium soaps of codliver and peanut oils as effective as that of chaulmoogra oil in inhibiting the growth of tubercle bacilli in cultures.

Twort and Ingram (1913) refer to observations made on the improvement in condition following the use of extracts of the bacillus for diagnostic purposes, and mention the desirability of experimental work on this phase of the disease.

The general results obtained by the use of tuberculin as a curative agent would seem to indicate that use of dead Johne's bacillus or extracts thereof would not prove of value. At any rate, the practicability of such treatment, whatever its effect should prove to be, is much in question.

Vaccination Against the Disease

Many types of vaccine have been used with the effort to protect animals

against tuberculosis. No practically valuable results have been obtained unless the plan suggested by Calmette, now being actively studied in a number of countries, should prove effective. The results which have been obtained in vaccinating against tuberculosis up to the present time would not lead one to believe that vaccination against Johne's disease is likely to be a procedure of value to the stock owners. Efforts, however, are being made experimentally to see what can be accomplished. Edwards (1926) is attempting the use of avian tubercle bacilli as a preventive vaccine. Vallée and Rinjard (1926) are also studying the question of vaccination against Johne's disease. Vallée uses a subcutaneous inoculation of five to 15 milligrams of the Johne's bacillus suspended in vaseline with which is also mixed some porphyritic grit, following the method suggested by Vallée (1923-24) for vaccination against tuberculosis.

Vallée believes that the introduction of such quantities of the bacillus beneath the skin has been shown by himself and others to have no harmful effect on the animal. Following the establishment of this fact on experimental animals, the work was carried to the field, where 277 animals to all appearances free from Johne's disease have been vaccinated. These animals are in herds known to be severely infected. Approximately one-third of the animals on the farms were left untreated to serve as controls. The treatment caused no trouble, and seems to have exerted a protective effect during the year following its use, at which time the preliminary report was prepared. On one farm three control animals showed evidences of Johne's disease, while the vaccinated animals remained free. On another farm where the controls became infected, one treated animal also showed evidence of infection. Vallée points out that the value of the treatment can be established only by observations on numerous animals over long periods of time.

Vaccination is not likely to be a subject which will be of interest or value to the American farmer who finds his herd infected with Johne's disease.

Eradication of the Disease

Eradication of the disease from a herd can probably be successfully accomplished only by the detection of the incipient cases through the use of johnin and by the removal of reacting animals from the herd. This does not, however, mean that something can not be accomplished by removal of animals showing symptoms of the disease.

The first step is the recognition of the disease in the herd. This can not be done by relying on symptoms, but only through the johnin test, supplemented by a post-mortem examination on a reacting animal or by post-mortem examination of an animal showing apparent symptoms. Diarrhea and emaciation may be due to such a variety of causes that one can not rely on them as conclusive evidence of Johne's disease. After the undoubted presence of the disease has been proven, however, the owner will probably not go far astray in removing all animals showing such symptoms. This will undoubtedly have some retarding influence on the progress of the disease in a herd, and it may have a very marked effect. Doctor Bang (1914) indicates that the removal of clinical cases is effective in preventing

the spread of the disease. Later (1921) in a private communication he states that the losses in Denmark have diminished within recent years due to the recognition of the disease by the farmers and to the voluntary removal of evident cases from the herds. The animals removed may be isolated from the herd, or, as probably will prove advantageous in most instances, be slaughtered.

The relative success which will follow the removal of clinical cases will depend on whether the elimination of the organism takes place prior to the appearance of symptoms or only late in the progress of the disease. Concerning this nothing is known.

The results of our work indicate that the disease may be eradicated from a herd by removal of all animals giving a positive response to johnin, or which show evident symptoms of the disease. The latter can be relied on as conclusive evidence of Johne's disease only when the presence of the disease in the herd has been proven by the johnin test or by post-mortem examination. As with the tuberculin test, no response may follow an injection of johnin in animals in the last stages of the disease.

The removal of apparently healthy animals which have reacted to johnin has not seemed wise to the owner in many cases unless his experience has shown him the necessity of doing something to prevent the excessive losses caused by the disease. The result has been that in only a few instances has a free opportunity been presented to us to test the value of johnin in eradicating Johne's disease.

In 1927 through congressional action the Federal Bureau of Animal Industry was permitted to work along the same lines as regards Johne's disease as with tuberculosis. This means that when any state provides means for eradicating the disease, that the Bureau of Animal Industry may cooperate with the state, and that federal funds may be used to pay a portion of the indemnity for reacting animals. In other words, the same plan of action as is now used in tuberculosis eradication work may be used with Johne's disease.

The legislature of Wisconsin in its 1927 session passed a law permitting the State Live Stock Sanitary Board to use $5,000 of the money appropriated for indemnities for tuberculous cattle as indemnity for cattle found affected with Johne's disease. The result of these actions will be that the farmer will see his way more clearly to the removal of animals which show no clinical symptoms, but which have reacted, and within a relatively short time sufficient evidence will have been gathered to show the effect of such a method in eradicating the disease.

Sanitary Precautions

The prompt removal of any animal in a known infected herd is undoubtedly a wise step in limiting the spread of the disease. The use of other means to limit the infection is more or less questionable, such as the employment of disinfectants, especially in view of the limited knowledge concerning the elimination of the organism by the affected animal.

There is little reason to believe that the disease can be introduced into

a herd other than through an infected animal. It may be that contact through fences might result in spread. It seems that the disease is much less easily acquired than is tuberculosis, and that the danger sometimes emphasized in the case of tuberculosis of infection by limited contact as in cars, stockyards, shows, etc., can scarcely be sufficient to cause any number of infections.

The protection of the herd must be based on the consideration of the animals introduced. Any animal from a diseased herd is a possible danger. Purchasers of breeding cattle should satisfy themselves as to the condition of the herd from which animals are to be acquired. The disease, as far as we are aware, is never considered by purchasers of stock at sales. Cattle are consigned to sales from herds known to be infected. With this disease, as with others, the farmer's chief protection must come from his own efforts, rather than from those of sanitary officials.

The greater danger at present is associated with the more valuable herds, since most of these have had much more opportunity to become infected than has the average farm herd.

Bibliography

Albien.—"The cultivation of the organism of chronic bovine enteritis." Berliner Tierärztliche Wochenschrift, Vol. 26, No. 41, pp. 793-794, 1910.

Andersen, C. W.—"Transmission of Paratuberculosis to Experimental Animals." Arch. Wiss. u. Prakt. Tierheilk. Vol. 47, No. 1, p. 77, 1921.

Angwin, J. T.—"Johne's disease in cattle." Proceedings Southern Counties Veterinary Assn., June 27, 1907.

————"Further notes on Johne's disease in cattle." Proceedings Southern Counties Veterinary Assn., May 14, 1912.

Bang, B.—Proceedings National Veterinary Assn., Liverpool, 1906-a.

————"Chronische pseudotuberkulöse Darmentzündung b i e m Rinde." Berliner Tierärztliche Wochenschrift, No. 42, S. 759, 1906-b.

————"Die spezifische chronische Enteritis des Rindes." Ninth International Veterinary Congress, Hague, p. 27, September, 1909.

————"Kvaegets smitsomme kroniske Tarmbetaendelse." 66 de Beretning fra den Kgl. Veterinaer-og Landbohoejskoles Laboratorium, p. 39 (Copenhagen Exp. Sta. Bul. 66, p. 39) 1910.

Bang, B., Lineaux E., Stuurman, W., Meissner, Markus, H., and Bongert, J.—"Der infektiose Darmkatarrh des Rindes." Ninth International Veterinary Congress, Hague, 1909. Same article is given in Vet. Jour., Vol. 65, No. 414, p. 608-614, 1909.

Bang, O.—"Das Geflügeltuberkulin als diagnostisches Mittel bei der chronischen pseudotuberkulösen Darmentzündung des Rindes" (Johne's Disease). Centralblatt für Bakt. I. (Originale), 51, pp. 450-455, 1909.

————"Om Anverdelse af Tuberkulin af Fjerkraetuberkelbaciller som diagnostisk Middel ved Kvaegets kron. smits. Tarmbetaendelse." 66 de Beretning fra den Kgl. Veterinaer-og Landbohoejskoles Laboratorium, pp. 41-50 (Copenhagen Exp. Sta. Bul. 66, pp. 41-50) 1910.

Bang, O. and Andersen, C. W.—"Einige Untersuchungen über Komplement bindende Antistoffe bei experimenteller u. spontaner Tuberkulose so wie bei paratuberkulöser Darmentzündung." Centralblatt für Bakt. Abt. I, (Originale) Vol. 69, pp. 517-538, 1913.

Beach, B. A. and Hastings, E. G.—"Johne's disease, a cattle menace." Bul. 343, Wisconsin Agr. Exp. Station, 1922.

————"Johne's disease and its detection." Jour. Infec. Diseases. Vol. 30, pp. 68-79, 1922.

Beebe, W. L.—"Johne's disease in cattle." American Veterinary Review, September 1908. Vol. 33, No. 6, pp. 709-716, 1908.

Bongert,—I. "Beiträge zur Lehre von der Entstehung der Tuberkulose." II. "Die tuberkulöse Infiltration der Darmmukosa beim Rinde, eine Form von Darmtuberkulose, welche durch abgeschwächte Säugetiertuberkelbazillen hervorgerufen wird." Deutsche Tierärztliche Wochenschrift, S. 229 and 241, Bd. 14, 1906.

Boquet, Par A.—"Sur les propriétés pathogénes du bacille de Johne (Enterite hypertrophiante des bovidés) a l'egard du rat blanc et de la souris blanche." Recueli de Méd. Vét. p. 741, Tome CI, No. 21, 1925.

Borgeand, A.—"Contribution á l'étude de l'entérite chronique des bovidés." Schweizer Archiv. Bd. 47, H. 5, pp. 226-229, (S. 569), 1905.

Bugge and Albien—"Vorläufige Mitteilung über die Enteritis chronica bovis pseudotuberculosa." Berliner Tierärztliche Wochenschrift. No. 10, p. 175, 1908.

Bugge, G.—and Cordsen, C.—"Einige Beobachtungen über die Enteritis chronica bovis pseudotuberculosa." Zeitschr. für Infektionskrankheiten der Haustiere. Bd. 5, S. 133-143, 1908.

Clark, D. B.—"Johne's disease." Proceedings American Veterinary Medical Association, Vol. 38, pp. 360-367. No. 3, 1910.

Claypoole, Edith J.—"On the classification of the streptothrices, particularly in their relation to bacteria." Jour. Exp. Med. Vol. 17, pp. 99-116, 1913.

Cruickshank, John—"On the direct cultivation of tubercle bacilli from tuberculous tissues." British Medical Journal, Vol. II, pp. 1298-1300, November 9, 1912.

Cumming, H. S.—"Tuberculin—a report on its standardization." American Review Tuberculosis, Vol. 14, pp. 1-57, 1926.

Dammann and Stedefeder—"Uber eine durch Pseudotuberkelbazillen hervorgerufene Pseudotuberkulose des Darmes der Kälber." Deutsche Tierärztliche Wochenschrift, No. 20, p. 296, 1910.

DeVine—"Johne's disease." Midland Counties Veterinary Assn., Proceedings, May 14, 1912.

Edwards, J. E.—"Johne's disease." Agr. Jour. India. Vol. 21, pp. 6-13, 1926.

Ernest, L. B.—"Recent developments in the control of Johne's disease." Jour. American Veterinary Medical Assn., Vol. 71, pp. 742-49, September, 1927.

Freger—"Entérite Chronique Hypertrophiante des Bovidés." Jour. de Méd. Vét. et de Zootechnie, Vol. 57, p. 287, 1906.

Gaiger, S. H.—"Scrapie." Jour. Comp. Path. and Therap., Vol. 37, pp. 259-277, 1924.

Hadley, Philip—"Microbic dissociation." Jour. Infect. Diseases, Vol. 41, pp. 1-312, 1927.

Hastings, E. G., Beach, B. A., and Hadley F. B.—"The detection of Johne's Disease by the use of johnin." Jour. American Vet. Med. Assn., Vol. 5, pp. 462-466, 1918.

Henley, R. R.—"The reaction and growth curves of cultures of tubercle bacilli." Am. Rev. Tuberculosis, Vol. 13, pp. 107-113, 1926.

Holth, H.—"Reinzuchtung des Bacillus der spezifischen chronischen Darmentzündung des Rindes (Paratuberklebazillus)." Zeitschr. für Infekt. Krankheiten u. Hyg. der Haustiere. Vol. 11, pp. 378-387, 1912.

Horne, H.—"Enteritis chronica pseudotuberculosa bovis oder die Johnesche Seuche Konstatiert in Norwegen." Norsk. Veterinaer, Tidsskrift, Vol. 20, No. 3, pp. 72-77, 1908.

————"The specific chronic enteritis of cattle (Johne's Disease)." Tidsskrift for det Norsk Landbrug, S. 139, 1908. S. 88, 1909.

————"Enteritis chronica pseudotuberculosa bovis." Berliner Tierärzliche Wochenschrift, No. 10, S. 193, 1909.

————"The specific chronic enteritis of cattle (Johne's Disease)." Berliner Tierärztliche Wochenschrift, 26, No. 5, pp. 109, 110, 1910.

Hurtrel D'Arboval—Dict. de med. et de Chir. Vet., Vol. 1, p. 516, 1826.

Johne and Frothingham—"Ein eigentümlicher Fall von Tuberkulose beim Rinde." Deutsche Zeitschr. für Tiermedizin und vergleich. Path., S. 438-54, Bd. 21, 1895.

Joshi, B. B.—"Report on Johne's disease." Poona Agr. Col. Mag., Vol. 11, pp. 85-89, 1919-20.

Koch, M., and Rabinowitsch, L.—"Die Tuberkulose der Vögel und ihre Beziehungen zur Säugetiertuberkulose." Virchow's Archiv. Beih. Z. Bd. 190, p. 246 (S. 448).

Krautstrunk, T.—"Impfung mit Vogeltuberkulin zur Erkennung der paratuberculösen Darmentzündung des Rindes." Zeitschr. für Infekt. Krankheiten der Haustiere. Vol. 20, pp. 267-286, 1920.

Leclainche—"L'Entérite Chronique Hypertrophiante des Bovidés." Revue Générale de Méd. Vét., p. 305, Tome 9, 1907.

Lienaux, E.—"Sur la Nature Tuberculeuse de l'Entérite Hypertrophiante diffuse du Boeuf; Nouvelles Experiences." Annales de Méd. Vét. No. 8, pp. 433-443, 1907.

Lienaux—"Tuberculosis in the horse." Vet. Record, April 19, 1913. (Abstract from Annales de Méd. Vét. 61e 1912, p. 653.)

Lienaux and Van Den Eeckhout—"Contribution á l'Étude d'une Entérite-Tuberculeuse speciale et de la Diarrhee chronique du Boeuf." Annales de Méd. Vét., Tome 54, p. 65, 1905.

Lindenburg and Pestana—"Inhibiting effect of chaulmoogra oil in the form of the sodium salts of the fatty acids on tubercle bacilli." Zeitschr. für Immunität. Bd. 32, p. 66.

Lissner, H. H.—"Chaulmoogra oil esters in the treatment of pulmonary tuberculosis." American Review of Tuberculosis, Vol. 7, pp. 257-263, 1923.

Ludwig—"Tuberkulöse Infiltration des Darmes bei einem Bullen." Sächs. Veterinärbericht, p. 82. 1904.

Lukens, R. M.—"Chaulmoogra oil in the treatment of tuberculous laryngitis." Jour. American Med. Assn., Vol. 78, No. 4, pp. 274-275.

M'Fadyean, John—"Johne's disease." Annual Report for 1907 of the Principal of the Royal Veterinary College, Jour. Royal Agr. Society of England, Vol. 68, pp. 205-206, 1907-a.

————"Johne's disease, a chronic bacterial enteritis of cattle." Jour. Comp. Path. and Therap., Vol. 7, pp. 48-60, 1907-b.

————"A case of Johne's disease successfully treated." Jour. Comp. Path. and Therap., Vol. 27, pp. 76-78, 1914-a.

————Annual report for 1914 of the Principal of the Royal Veterinary College. (Deals with the occurrence of Johne's Disease.) Jour. Royal Agr. Society of England, Vol. 75, pp. 252-269, 1914-b.

————"The histology of the lesions of Johne's disease." Jour. Comp. Path. and Therap., Vol. 31, pp. 73-87, 1918.

M'Fadyean, John, and Sheather, A. L.—"The experimental transmission of Johne's disease to cattle, sheep and goats." Jour. Comp. Path. and Therap., Vol. 29, pp. 62-94, 1916.

M'Fadyean, John, Sheather, A. L., and Edwards, J. L.—"Johne's disease: (1) Records of examinations of natural cases; (2) Cultural characters of the Bacilli." Jour. Comp. Path. and Therap., Vol. 25, pp. 217-275, 1912.

————"The treatment of Johne's disease." Jour. Comp. Path. and Therap. Vol. 28, pp. 196-216, 1915.

————"The reaction of animals to johnin (Johne's Disease)." Jour. Comp. Path. and Therap., Vol. 29, pp. 134-171, 201-243, 1916.

Male, G. P.—"The clinical aspects of Johne's disease and the avian tuberculin test." Vet. Record 23, No. 1192, pp. 723-726, 1911.

Malm, O.—"Om den specifike kroniske tarmbetaendelse hos kvaeget." Norsk Veterinaer-Tidsskrift, No. 8, pp. 209-227, 1911-a.

————Medicinsk Revue (Bergen) 1911-b.

————Referat. Deutsche Tierärztliche Wochenschrift. No. 37, 1912.

Manuel, J. B.—"The treatment of Johne's disease." Jour. Comp. Path. and Therap., Vol. 27, pp. 172-173, 1914.

Markus, H.—"En Specifieke Darmontsteking bij het Rund, Waarschijnilijk van Tuberkuleusen aard." Tijdschrift voor Veeartsenijkunde Maandblad, Deel 30, No. 5, pp. 195-206, 1903.

————"Eine Spezifische Darmentzündung des Rindes wahrscheinlich tuberkulöser Natur." Zeitschr. für Tiermedizin, Bd. 8, S. 68, 1904.

Mathis—"Lesions de la Diarrhée chronique des Bovidés." Bulletin de la Soc. des Scien. Vét. de Lyon. No. 2, 1906.

Mellon, B. H. and MacGinnis, P.—"Chronic diarrhea in cattle (Johnes Disease)." Jour. Dept. Agr. and Tech. Inst., Ireland, No. 2, Vol. 13, pp. 311-324, 1913.

Melvin, A. D.—"Chronic bacterial dysentery." 26th Annual Report, Bureau of Animal Industry, pp. 37-38, 1909.

Meyer, K. F.—"Über die durch säurefeste Bakterien hervorgerufene diffuse Hypertrophie der Darmschleimhaut des Rindes." Jena, 1908, pp. 107.

Arbeiten aus dem Inst. zur Erforschung, der Infekskr. in Bern aus den Laborat. des Schweizer. Serum u. Impf. Inst., Heft 2, 1908. Reviewed in Gén. Méd. Vét. No. 170, pp. 91-93, 1910.

————"Chronic bacterial dysentery." Annual Report of the Penna. Dept. of Agr., p. 129, 1911.

————"Bacillus para-tuberculosis Johne." Proceedings Philadelphia Path. Society, October, 1912.

————"The specific paratuberculous enteritis of cattle in America." Jour. Med. Research Vol. 29, pp. 147-198, 1913.

————"Zur chronischen, paratuberkulösen Darmentzündung des Rindes." Schweizer Archiv. für Tierheilkunde, pp. 393-403, 1914.

Miessner and Kohlstock—"Immunisierungsversuche beim chronischen Infektiösen Darmkatarrh." Berliner Tierärztliche Wochenschrift No. 25, pp. 450-452, 1912.

Miessner and Trapp—"Der Chronische Infektiöse Darmkatarrh des Rindes." Mitt. d. Kaiser Wilh. Inst. für Landwirtschr. in Bromberg, 2, S. 219-286, 1910.

Mohler, J. R.—"Chronic bacterial dysentery of cattle." Circular 156, United States Bureau Animal Industry, pp. 1-3, 1908.

————"Chronic bacterial dysentery." 27th Annual Report of the Bureau of Animal Industry of the U. S., pp. 80, 1910.

Moore, V. A.—"Factors involved in tuberculosis eradication and in maintaining sound herds." Cornell Vet., Vol. 14, pp. 275-285, 1924.

Morin, H. and Valtis, J.—"Sur la filtration du bacille de Johne a travers les bougies. Chamberland L 2," Compt. R. Soc. de Biol. Vol. 94, p. 39, 1926.

Morris, Harry—"Johne's disease—Infectious diarrhea of cattle." Louisiana Agr. Exp. Sta. Bul. 200, June, 1927.

Moussu and Faroy—"Johne's disease." Compt. R. de la Soc. Biol. Vol. 70, pp. 938-940, 1911.

Pearson, Leonard—"Bacterial dysentery." Annual Report of the Penna. Dept. of Agr., p. 109, 1907.

————"A note on the occurrence in America of chronic bacterial dysentery of cattle. American Vet. Review, Vol. 32, No. 5, pp. 602-605, 1908.

Penberthy, J.—"Johne's disease." Jour. of Bath and West and Southern Counties Society. Vol. VII, pp. 1-11, 1912-13.

Plum, N.—"Geflügeltuberkulose bei Saugtieren." Den Kongelige Veterinaer- og Landbohoejokole Aarsskrift, pp. 63-185, 1925.

Rieck, M.—"Eigentümliche Darmtuberkulose bei einem Rinde." Bericht über das Veterinärwesen im Kgr. Sachs, p. 118, 1896.

Rogers, Leonard—"Preliminary note on the use of gynocardates orally and subcutaneously in leprosy." Lancet 94-1, pp. 288-290, 1916.

Rogers, Leonard and Muir, Ernest—"Leprosy." P. 257-274, 1925.

Romberg, E.—"Zur Serumdiagnose der Tuberkulose." Deutsche Med. Wochensche. Nos. 18, 19, pp. 273, 292, (S. 350), 1901.

Salvisberg—"Johne's disease." Schweiz. Archive für Tierheilkunde, Vol. 56, p. 196.

Schalk, A. F.—"Experiments on transmission of avian tuberculosis to cattle by cohabitation." Bul. 194, North Dakota Exp. Station, 1926.

Schmaltz—Berliner Tierärztliche Wochenschrift No. 1, 1909.

Schmidt, F. M. and Pröscholdt, O.—"The use of antiformin for detecting the open forms of tuberculosis." Zeitschr. für Infektionskr. Hygiene der Haustiere, xi, Heft. 5, pp. 321-364, Heft. 6, pp. 401-449, 1912.

Schutze, Harry and Zilva, S. S.—"Diet and Tuberculosis." Jour. of Hygiene, Vol. 26, pp. 204-212, 1927.

Sheather, A. L.—"Johne's disease." Vet. Record, Vol. 27, pp. 276-281, 1914.

————"Johne's disease." Agr. Jour. India. Vol. 13, pp. 23-31, 1918.

Stockman, Stewart—"Johne's disease." Report of Chief Veterinary officer to the Board of Agr., Board of Agr. and Fisheries, Annual Reports and Proceedings, p. 23, 1909.

————"Johne's disease (scrapie) in sheep." Report of Chief Veterinary officer to the Board of Agr., Board of Agr. and Fisheries, Annual Reports and Proceedings, pp. 18-19, 1911.

————"Johne's disease in sheep." Jour. Comp. Path. and Therap. Vol. 24, pp. 66-69, 1911.

Townsend, C. W.—"Johne's disease in cattle." Proceedings Lincolnshire Veterinary Assn., No. 1094, pp. 870-872, 1909.

Twort, C. C.—"The agglutination and complement fixation reactions in animals experimentally injected with Johne's bacillus." Centralblatt für Bakt. II, Bd. 66, pp. 316-320, 1912.

————"The rapidity with which contamination of the thoracic cavity and its contained glands follows infection of the peritoneal cavity." Lancet 91-2, pp. 216-218, 1913-a.

————"The experimental production of Johne's disease in some of the laboratory animals." The Veterinary News, June 14, 1913-b. The Veterinary News, Jan. 24, 1914.

————"Studies on immunity to tubercular diseases. 1. The caseation of the tissues." Vet. Jour., Vol. 70, pp. 543-551, 1914.

Twort, C. C. and Craig, T.—"The pathogenicity of Johne's bacillus compared with that of other acid-fast bacilli for some of the laboratory animals." Centralblatt für Bakt. I Abt. (Originale) Vol. 68, pp. 455-470, 1913.

Twort, F. W.—"A method for growing and isolating the lepra bacillus of man." Proceedings of the Royal Society, Vol. 83, September, 1910-a.

————"Cultivation of the acid-fast bacillus found in the intestine of cows in Johne's disease." Proceedings of the Royal Society, Ser. B. Vol. 83, No. B 562, p. 158, 1910-b.

————"Affection of the bronchial glands in Johne's disease." The Veterinary News, June 21, 1913.

Twort, F. W. and Ingram, G. L. Y.—"Further experiments with the Mycobacterium enteritidis chronicae pseudotuberculosae bovis, Johne, and with vaccines prepared from this micro-organism." Centralblatt für. Bakt. 1 Abt. (Originale) Vol. 67, pp. 126-135, 1912-a.

Twort, F. W. and Ingram, G. L. Y.—"A method for isolating and cultivating the Mycobacterium enteritidis chronicae pseudo-tuberculosae bovis, Johne." Royal Soc. Proceedings, Vol. 84, No. B 575, pp. 517-542, 1912-b.

————"Some further researches on Johne's disease." Vet. Jour. No. 448, pp. 569-572, 1912-c.
————"A monograph on Johne's disease." London, 1913-a.
————"Johne's disease in sheep." Vet. Record. No. 25, p. 635, 1913-b.
————"Further experiments on the biology of Johne's bacillus." Centralblatt für Bakt. Vol. 73, Abt. 1 (Originale), pp. 277-283, 1914.
Vallée, H.—Bacille tuberculeaux et excipient irresorbable." Comptes Rendus de l'Academie des Sciences. December 26, 1923.
Vallée, H. and Rinjard, P.—"Etudes sur l'enterite paratuberculeuse des bovidés." Revue Générale de Médecine Véterinaire. Jan. 15, 1926.
Van Der Sluys—Generalversammlung der Niederländischen Tierärzte in Utrecht, September, 1920.
Walker, E. L. and Sweeney, Marion A.—"Chemotherapeutics of the chaulmoogric acid series and other fatty acids in leprosy and tuberculosis." Jour. Inf. Diseases, Vol. 26, p. 238, 1920.
Wilkins, V. C.—"Research and the Land." A report of recent progress on agricultural science in the United Kingdom, p. 278, 1926.
Wills, J. G.—"Johne's disease or pseudo-tuberculosis." Rept. 16th Annual meeting U. S. Livestock Sanitary Association, pp. 44-50, 1912.
Wolff-Eisner, A.—"Frühdiagnose und Tuberkulose-Immunität." 378 S. Wurzburg, 1909. Reviewed in Centralblatt für Bakt. Abt. 1 (Referate) Vol. 45, p. 286, 1910.

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