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Epidemiology of John's Disease, a Review

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Abstract:

In last decades, the prevalence and importance of Johne's disease is increased, and consequently different programs are designed for its control. Johne's disease is considered the most important disease of cattle industry regarding the economic lost. In addition they are published several data, which implicate the *Mycobacterium avium* subsp. *paratuberculosis* as a pathogen bacteria of Chrohn's disease in humans. The chronic pattern of disease, complexity of its path biology, limitations knowledge's on pathogenesis, poor sensitivity and specificity of currently available laboratory diagnostic methods, lack of successful vaccines, make Johne's disease control a significant challenge for veterinary services and scientific community as well. This paper aim to highlight the current achievements on path-biology, diagnosis, biosecurity, in order to translate them in practical action for Johne's disease control.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*, Johne's disease, INF-γ, Disease control, Paratuberculosis.

1. Introduction

John's disease is defined as chronic, contagious, invariably fatal enteritis which can affect domestic and wild ruminants due by *Mycobacterium avium* subsp. *paratuberculosis* [12, 15].

Despite that a chronic emaciated disease was describe at early 1826 as intestinal disease of cattle, nothing was known till 1894 regarding the etiology and disease itself. The name of John's disease is related with a sick caw in Oldenburg region of Germany (1894), that died in spring 1895 when local veterinarian Herr Frederick Harmes diagnosed as suspected intestinal tuberculosis. The pathogen agent was evidenced by Dr. H.A John in Veterinary Pathology Unit, Dresden Germany and Dr. L. Frothingham, a visiting scientist from the Pathology Unit in Boston, Massachusetts who both examined the intestinal tissues, mesenteric lymph nodes and detected abundant acid-fast bacteria. They concluded that pathogen agent was *Mycobacterium avium* and proposed the name pseudotuberculosis for disease, based on similarity to intestinal tuberculosis lesions, caused by *Mycobacterium bovis*. The disease was named as "John's disease" in 1906, in the Annual Report for 1906 of the Principal of the Royal Veterinary College. John's disease in USA was described in 1908 by L.Pirson, in the 1920s, was described in Africa and Asia, and during 1930 was described in South America and on the Indian continent, thereby completing evidence of the worldwide distribution of the disease. In the 1940s, paratuberculosis disease was recognized to be a problem not only in domesticated livestock, but in wildlife as well.

In 1913, appear the first reports on economic importance of John's disease, and in 1922 were publish the relevant advises for farmers to stop the disease. Many studies are done in different countries worldwide about epidemiology of the disease and significant progress was made in the last ten years in understanding the entire biology complex of John's disease.

Despite significant achievements and substantial investments, again the John's disease remains a major problem in ruminant animals. The best example of this judgment could be USA experiences, where there are reported more than $100 million expenses, but John's disease prevalence has been increased from 22% to 68% in almost last 10 years [11]. The prevalence of Map in Europe is increasing year by year, however there are only a few studies available to estimate the real prevalence. According to Nielsen, the true prevalence of Map among cattle in Europe is 20%, and between herds the prevalence is estimated > 50% [15]. The first clinical cases, confirmed by microscopic slide examination, of Map in Albania dated in early 1970 [18]. A longitudinal study carry out in five dairy cattle Albanian herds shown a

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positive Map evidence range from 21 to 31%, with a average 24.73% [18]

John's disease is spread worldwide and affects a range of animal species. It is with high importance for domestic ruminants, in particular for cattle. Among all other infectious disease, paratuberculosis, has the most negative economic impact in dairy cattle [11]. In last decade, many efforts are focused in better understanding the complex biology of Map and possible implication of *Mycobacterium avium* subsp. *paratuberculosis* (Map) in Crohn's disease in human [2, 5].

2. Epidemiology

The pathogen agent of John's disease is *Mycobacterium avium* subsp. *paratuberculosis*, an aerobic, non-spore forming, non – motile, acid-fast bacilli. Map is an intracellular growing bacterium; it causes a typical chronic granulomatous local inflammation which is very difficult to diagnosed [12].

The main sources of infection are colostrums and milk of infected animals, the contaminated environment by feces [11]. The lesser important sources of infection are genital organs and semen [12]. Despite the Map can survive up to a year in environment it is not capable to multiply there, so the number of viable Maps will be reduced over time [11]. Milk is an important source of Maps, even after pasteurization. There are some evidences showed that Map could survive in hard cheese for twelve days [11].

Biology of Map is very complex and not well full understands yet. Based on best knowledge's authors, Map infection could be an excellent example of iceberg pattern of infection. In order to have a better picture of John's disease we will try to explain in further details the risk factors which are responsible for disease itself and have a highly contribute in Map infection management and its control.

3. Occurrence

Johne's disease is spread in all over the world [11]. Paratuberculosis have been reported in farm animals, captive and free-ranging wildlife, however the infection is most prevalent in domestic ruminants. Johne's disease prevalence is very variable and the latest decade reports shown a continuously increasing in both herd and individual levels. The management system, the high animal density, concentration of Map in colostrums, milk and environment and existence of many way of transmission explain the higher prevalence of Map infection in domestic animal. Cattle sero-prevalence between-herds in USA is reported to be over 50% [11], while in Europe was guesstimates >50% [15]. In other affected animal species there is little information and clinical manifestations actually almost missed.

4. Risk factors

Host range

The natural hosts of Map are all ruminant animals, however most affected species are cattle [11, 12]. Despite the John's disease has a specific etiology there are several factors that affect its pattern. The risk factors are related to the agent, host, environment and management. ***Ruminant*** species are the natural host and most affected by Map. As a general rule, in cattle, sheep, goats, bison, moufflon addax, eland and muntjac, infection proceeds to gastrointestinal disease and eventually death. ***Non-ruminant*** species are considered as atypical host; however a few evidences are described in fox, stoat, badger, and raven. In addition, despite absence of insufficient completed studies, it is assumed that those atypical species are "dead-end" hosts, and are considered without any epidemiological significance either as pathogen shedders, transmission and or reservoirs of infection [11, 15]. Horses, pigs, and nonhuman primates could be affected but their clinical and epidemiological importance is not well established.

5. Transmission of infection

As it is represent in Diagram 1 the main cicle transmission of MAP occurs from adult infected animals to young offspring through the fecal-oral route [11, 12. 13, 18]. The organism is swallowed in manure-contaminated milk, water or feed. MAP is also shed directly into the milk and colostrums of infected dams in later stages of infection, providing another route of exposure for susceptible young animals [11]. The likelihood of MAPs transmission, after infection is established into the herd, is greater in twins or triplets (sheep and goats) than single offspring [11, 13]. Transplacental transmission is another important route, where fetuses acquired the MAP infection; even it is demonstrated that embryo transfer technology is not safety method for prevention of vertical transmission [11]. There are no evidences of MAP transmission by direct contacts nose-to nose or aerosols. Not every exposure to MAP necessary established the infection and eventually

clinical disease. The artificial insemination or natural breeding, epidemiologically, are consider as less important routes of transmission as far as isolation of MAP from bulls and rams semen was confirmed only one cases respectively [11, 12]. It is important to emphasize that the way of MAP infection introducing in free flocks/herds are the carriers' animals which are shedder of pathogens, and inside herd the infection transmission occur by exposure of new generation to contaminated milk, colostrums, dirty environment, in particular birthing place, contaminated water and food [2, 11, 12, 13, 18].

6. Pathogenesis

Pathogenesis of Johne's disease is very complex and despite remarkable research work is not fully understood yet (Diagram 1). There are some unanswered scientific questions which interfere with understanding the epidemiology of Johne's disease which affect successful control programs, including availability of successful vaccine for susceptible species. We don't know: minimally infective dose for different species and different ages; the reasons why young ruminants animals are much more susceptible than adult ruminants; possibility of recovering of animals after naturally infected, if yes how is the proportion of recovering; the exact virulence factors; existence of difference on virulence between MAP stains; the pattern of immune response in young animals. The MAPs have a preference for gastrointestinal tract tissues and M cells in intestinal Payer patches uptake the pathogen. The tonsils and retropharyngeal glands may be exposed, however they are lesser involved in the pathogenesis of disease. The fibronectin binding proteins play an important role in bacteria attachment and after it cross the epithelial layer, the macrophages uptake the pathogen [6]. The intracellular surviving ability is mediated by virulence factors which prevent phagosome-lysosome fussion [6]. There are described three types of animals regarding the host pathogen interaction; 1) resistant animals, consequently those animals are not important as MAP shedders, 2) moderate resistant animals, infection partially could be controlled, and animals of this group shed the bacteria intermittently, 3) susceptible animals, animals from this group could be heavy shedders and clinical cases will occur [11]. The MAPs could persist for long time inside macrophages without any significant visible immune-response, however those animals could be either continuously or intermittently shedders. The incubation period is very variable; in general it is over 2 years, and only a small percentage of infected animals manifest clinical disease. The clinical Johne's disease have fatal course; however it is not systemic disease. During the first stage of Map infection a primary immune response based on activating Th1 cells and production proinflammatory cytokines, while the humoral response happen in advanced clinical cases. At the begging phase, the inflammatory response is switch of by MAP (mechanisms are not known), and no any visible lesion appeared. Later during infection, the primary response happens, macrophages recruit continuously and different cytokines are involved, the most important is INF-γ. The time that detectable antibody anti MAP appeared in sera is not precise, but as general rule they rich the threshold late after infection, close to the time that clinical signs start [20]. Most invading tissue by MAP is terminal part of ileum an ileocecal valve [11, 12]. As MAPs replicate more macrophage recruit severe granulomatous inflammation progress and spread to deeper intestinal layers, surrounded tissue, regional lymph nodes and expanded to blood and consequently in other organs systems. In advanced stage of infection the gastrointestinal tract is severely damaged and is no longer capable o ply its functions. The proportion of animal which show clinical disease is low and most of animals do not show typical clinical signs. The clinical signs of Paratuberculosis become evident only in advanced stage, in cattle at age 2 years, in deer at age 1 year, only in mature sheep and goats [23]. Clinically affected animals suffer from diarrhea or weight loss, the intestine is usually thickened, corrugated and reddened; regional lymph nodes are swollen and pale.

7. Control

Control of Johne's disease is a very complicated and difficult issue (Table 1 and Diagram 2). The approaching methodology for MAP controlling design, are quite similar to other infectious disease control, however the details are not the same. The successful vaccines against MAP are not available yet. The control of MAP infection is based on strict applying of biosecurity measures, sanitation program, manure management, disinfection, milk and colostrums management, animal identification, movement animal control, surveillance program, and based on using proper diagnostic tests.

The confirmation of MAP infection could be made only by laboratory diagnosis. Laboratory methods employed for MAP diagnosis are summarized in Table 1. Each laboratory diagnostic

tests used for MAP have their limitations, and using two or more different tests in combination either in parallel or series could improve sensitivity and specificity, respectively. The results of diagnostic tests are useful for screening of the herd, estimation the prevalence, evaluation of the disease control program, confirmation the diagnosis for sick animals, testing the environment for presence of MAP, fulfilling the requirements for purchasing purpose. In order to control the Johne's disease it is important to design the surveillance program based on needs, test availability, prevalence of disease, and aim of owner. The program must described the details for type of test, the period for performing the test, defining the animal categories, cost of the test, methodology for interpretation the results of the test. In general, there are two main types of diagnostic tests; 1) tests that identify the pathogen agent, *Mycobacterium avium* subsp. *paratuberculosis* and 2) tests that identify immunoresponse of the host, either cell mediated or humoral mediated immunity. The laboratory test can be performed in samples originated from live and dead animals. Proper samples from live animals are feces, rectal scraping, rectal biopsy, mesenteric lymph nodes biopsy, blood and sera. The samples from dead or slaughtered animals are ileum, ileocaecal valve and mesenteric lymph nodes. Path biology of Johne's disease is very complex and not fully understood. Those limitations partly explain the difficulties for successful Johne's disease control.

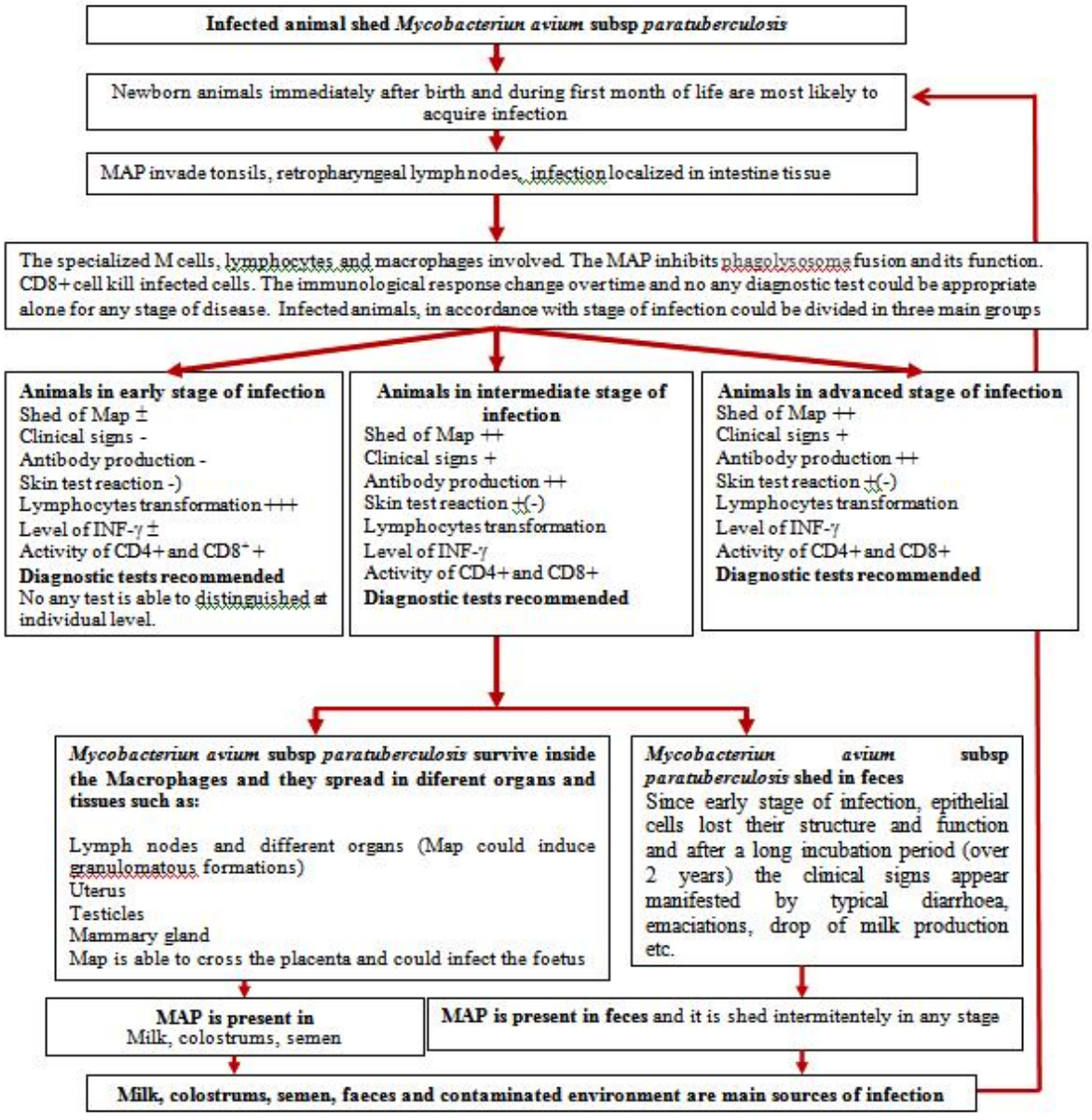

Diagram 1 Proposed pathogenesis of Johne's disease in cattle

Table 1. Summary of laboratory tests used for Johne's disease diagnosis, and relevant comments

Test method	Comments
Tests based on evidence of *Mycobacterium avium* subsp. *paratuberculosis*	
1. Microscopic examination	The test could be performed in feces, Rectum scraping live, rectum biopsy, mesenteric lymph nodes biopsy of live animals. The direct microscopic examination of feces stained by Ziehl-Neelsen methods have limited sensitivity and could identify up to 25% infected shedders. It is believed that positive animals are heavy shedders and clinical signs will be appear in six to three years after. In order to increase the test sensitivity, test repetition at regularly interval is recommended [1, 11, 12, 17].
2. Culturing by using Herrold's egg yolk medium	The method is better than microscopic examination and it has high specificity, from 98 - 100%. However it suffer from some important limitation: it has high cot, usually it is performed in reference laboratory, samples prior culturing need to be decontaminated, need special media (addition of amphotericine B as antifungal contamination, and Mycobactine J as grow factor), culturing in quarto plate for each samples (three with and one without Mycobactine J), the test results will be available to late (8-16 week or even more), and the test has a low sensitivity (which range from 25% to 75% for infected animal and clinically animal respectively). Despite limitation, still it remains gold standard test and individual animals. Culturing methods are effective for testing any species, manure and tissue samples, in addition environmental samples soil, water, grass, etc., may also be tested by culture. In order to reduce the cost for herd test the pooling manure samples are recommended to be tested first and positive ones could be tested later on.
3. Culturing in liquid media	BACT technology is an relatively new approach for MAP culturing based on liquid media culturing and its MAP identification on radiometric and fluorimetric method. The advantages of these methods are: the positive samples could be identifying at 29-35 day after cultivation; and the cost for samples is lower. This method is recommended for Johne's disease control program in a large scale. Disadvantages of this approach could be: firstly establishing the laboratory equipments have a high cost, it is not suitable for small number of samples; the test sensitivity remains lower despite is higher than conventional culturing method. Identification of isolates is based on colonial morphology, microscopic appearance and Mycobactine dependence [1].
Molecular Methods	
1. Conventional PCR	This method is based on detection of IS900 and is specific for *Mycobacterium avium* subsp. *paratuberculosis.* The PCR method is recommended as confirmatory test for isolates confirmatory. This is validated method for cattle only.
2. Real Time -PCR Methods (RT-PCR)	RT-PCR Methods are available for detection of MAP in feces and tissues and are comparable with culturing method in regards of their sensitivity. The results will obtained in very short time compare with culturing assays.
Field test	
1. Intradermal Johnin test	The principle of the test is based on delayed hypersensitivity reaction and is available for cattle only. The results, as for bovine tuberculosis could be read 48-72 hours after injection, and presence of reaction at injection site serve as indication of positive results. The main limitations are false- positive results, which could be up to as high as 3/4 of animal tested.
2. Intravenous Johnin test	The positive results are based on elevation of temperature (1.5^0C) after intravenous injection of Johnin. This test is more sensitive than Intradermal test and could detect 80% of clinical cases. The limitations include: it is not available for other species except cattle, it is not able to detect salient shedders, risk for anaphylactic shock, time consuming.
Immunological test (In vitro lymphocyte stimulation and serological tests)	
1. In vitro lymphocyte stimulation	The test is based on exposure of peripheral blood lymphocytes to johnin. Proliferation of lymphocytes will occur if the blood belongs to infected animal. The usefulness of the test is limited as positive animals are considered only if clinical signs are visible.
Serological tests	
1. Complement fixation test	Specificity of CFT is comparable to other serological tests, while the sensitivity is low. The several studies shown that ELISA tests are superior to CFT and for either certification or diagnosis confirmation.
2. Agar gel immunodiffusion test (AGID)	AGID is designed for detecting the antibody in blood. It can be used in cattle, sheep, goats. The AGID has low sensitivity and specificity rates, it is unsatisfactory as a screening diagnostic method for subclinical herd infection.
3. Indirect Fluorescent antibody microscopy test (IFAMT)	Results of IFAMT are not significantly different from complement-fixation test results in diagnosing paratuberculosis (Johne's disease) in cattle. IFAMT is not suitable for detecting subclinical animal. Both, IFAMT sensitivity and specificity are unsatisfactory for screening or confirmatory purpose.
3. Immunoperoxida-	IPT is more sensitive than ZN stain. It is an useful test for confirmatory diagnosis and research

-se tests (IPT)	purpose for pathogenesis. The main limitation is relating with its use in samples take in necropsy.
4. Gama interferon test (γ-INF) test	The γ-INF test evaluate the cell mediated immunity, it is not properly validated for large scale using. The γ-INF test detect the infected animal in subclinical cases, much earlier than other serological test, including ELISA methods. False-positive results are common. It could be recommended as an appropriate test for use as a supportive tool for evaluation of disease-preventive measures.
5. ELISA methods	There are developed different ELISA methods. Their principal work is based on detecting of antibody in blood and milk at individual animal level, and is designed for testing large numbers of samples with low-cost. The test results obtained in a few days and is validated for cattle, sheep, goats and deer in some countries. The commercially ELISA tests (not all ELISA) are validated and available for using in cattle, sheep, goat and deer. There are recommended as screening test and employed on different control program. The main limitations of ELISA methods are; ELISA is not suitable for testing young animals, overall low sensitivity (especially at early stages of infection), high number of false positive results, and they are not suitable for confirmatory diagnosis.

It is important to emphasize that despite the infection most likely occur during first month after birth, no any available laboratory diagnostic test is able to detect infected animals even in young adult animal. The target animals for testing are cattle at least 18 months old, sheep and goats over one year animals.

Paratuberculosis, despite its complexity and limitation of diagnostic tests, could be controlled even it could be eliminated from flocks. The control strategies for Johne's disease depend on epizootological status.

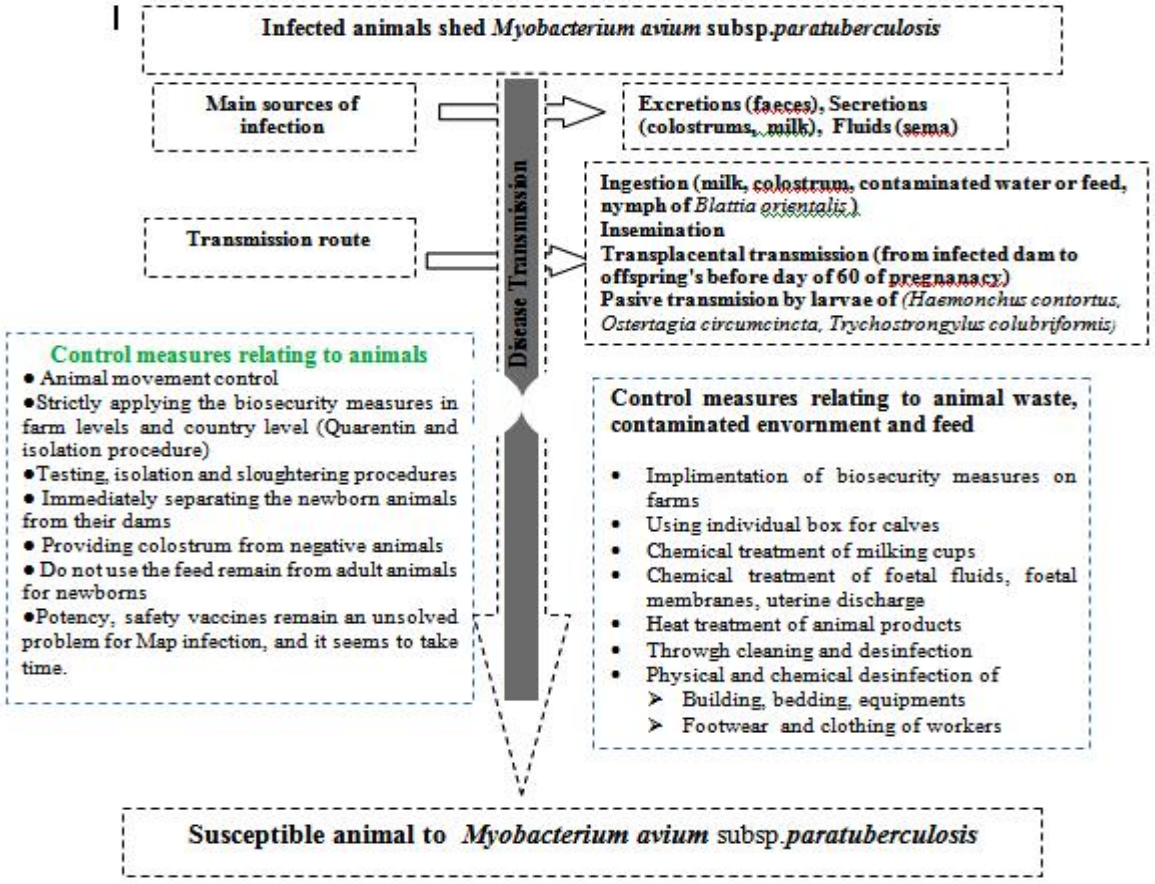

Diagram 2. Epidemiological scheme of Johne's disease control

If the region, herds or flock that are free of disease, management as close herds is essential for keeping the status. Biosecurity measures include; choosing the trusted sources for purchasing new replacement animals, applying isolation procedures in the farm for new entry animals for at least two weeks and testing them for presence of disease, avoiding grassing in contaminated pasture, avoiding sharing of equipments between herds.

If the disease is present, it is important designing a proper control program. The program includes close consultation with owner, veterinarian and requires use of one or more of the available diagnostic tests. The effective control program last over five years.

There are two main components of the program: preventing of new infection and identification and removing the infected animal.

Preventive measures in the infected herds include: keeping clean calving, lambing and kidding area, keeping the newborn animals separated from adults animals. Sanitation and manure management are essential for MAP infection control. Newborn animals from infected mothers must be feed with uncontaminated milk and colostrums, pasteurized milk and colostrums at 66^0C for 30 minutes, or artificial milk replacers. Thoroughly cleaning the udder and teats before collection of colostrums to avoid manure contamination.

The strategy based on test and slaughter program is essential to successful control of Johne's disease in herds or flocks in a reasonable period of time. In some situations there are alternative methods which must be considered. The program based on testing and culling of all test-positive animals is not suitable for many situation. With the great diversity of animal species affected by Johne's disease and the differences in husbandry practices and economic value of these different species, it is difficult to generalize about test-and-cull recommendations. Animal identification and breeding records are important for follow up the disease status

Decontamination of environment could be achieved by applying disinfection to MAP. The MAP is resistant to most disinfectants; washable tools, troughs, and feed dishes may be treated as directed on the bottle with a disinfectant labeled as "tuberculocidal". Since organic material deactivates the disinfectant, items should thoroughly cleaned with soap and water, rinsed and dried before the disinfectant is applied. Tuberculocidal disinfectants usually contain strong chemical compounds and should be used carefully. The instructions provided on the label for proper use and safe handling should be followed precisely

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