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Review Paper

PARATUBERCULOSIS (JOHNE´S DISEASE) IN RUMINANTS – AN ONGOING STORY

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Summary: Paratuberculosis or JOHNE´s disease is one of the most important diseases in ruminants today. Mycobacterium avium subspecies paratuberculosis (MAP), the cause of paratuberculosis, has a broad host range. Although mainly considered a disease of cattle, sheep and goats, all ruminants, including a large number of wild and exotic species of ruminants, are susceptible. The reported prevalence of infected animals varies by country and region and reaches up to 84.7 % MAP positive dairy herds in some aereas. Although calves are most susceptible to paratuberculosis, older heifers and adult cattle can also become infected. In newborn calves the infection mostly takes place soon after birth by oral ingestion of the organism but MAP can also be found in colostrum and milk of asymptomatic infected cows. Although shedding of MAP is considered not to start before 1.5 years of age, it has been shown, that also young calves can shed MAP and thereby pass infection to other calves in the same environment. The vast majority of herds that acquire MAP do so through purchase of infected animals. The first clinical sign in cattle can usually be seen after calving as a chronic or intermittent diarrhea alternating with periods of normal consistency of faces. In sheep and goats diarrhea is usually absent.

Subclinically infected adult animals do not show visible signs of paratuberculosis, although they carry MAP. The prognosis is poor, the disease leads to emaciation and ends with the death of the affected animal. If clinical symptoms are missing laboratory tests have to be used to confirm or rule out the diagnosis. Many different test systems are available for the detection of MAP, such as Ziehl-Neelsen Staining, faecal culture and Polymerase Chain Reaction (PCR) or the evaluation of antibody levels by Enzyme Linked Immuno Sorbent Essays (ELISA).

Because paratuberculosis is difficult to diagnose and untreatable control and reduction in MAP positive herds and prevention of spreading the disease to negative herds is very important. Hygienic precautionary measures have to be taken to prevent further spreading of the disease in a herd. The mayor aim of this hygienic precaution is to prevent infection of calves and young stock and to purchase MAP- free animals only.

In this article a review about infection, diagnosis and control of this disease is given. New aspects of current researches are combined with basic informations to provide detailed and actual information about this infectious disease for large animal veterinarians.

Keywords: cattle diseases; paratuberculosis – diagnosis – epidemiology – preventive and control; *Mycobacterium paratuberculosis* – pathogenicity; cattle; sheep

History and Etiology

It was in 1895 that Johne and Frotingham (1) first described the disease and demonstrated the presence of acid-fast bacilli in sections of the intestine of infected animals. The presence of these bacilli, indistinguishable from the tubercle bacillus, made them think the condition was an atypical or unusual form of tuberculosis. More than ten years later, in 1906, Bang (2) determined it was not tuberculosis and called it pseudotuberculous enteritis. The disease later became known as paratuberculosis or JOHNE´s disease.

Paratuberculose is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a slow growing, acid fast bacillus dependent on mycobactin containing culture media (3). The

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organism can remain viable for more than 1 year in bovine faeces and black soil, 9 months in pond water and about 160 days in river water. Temperatures of -14°C can be survived for at least one year. Phenolic or cresylic agents have to be used for disinfection. Products labelled as tuberculocidal are also effective against MAP.

Host range

MAP, the cause of paratuberculosis, has a broad host range. Although mainly considered a disease of cattle, sheep and goats, all ruminants are susceptible. Paratuberculosis has been reported in a large number of wild and exotic ruminants such as deer and mouflon (4), water buffalo (5), and camelids (3). Several reports also describe infections in non-ruminant species like different kind of birds, wild boar (6), mice, rats, hares and fox (7), rabbits (8), bears, different insects and earthworms (9). Pigs and primates (10) are also known to carry MAP, but, as all other monogastriers, do not suffer from clinical signs of paratuberculosis. There are also infrequent reports of infections and increased incidence of specific antibodies against MAP in humans suffering from Morbus CROHN, leading to ongoing discussions about a possible connection between the two diseases (11, 12).

Occurence

Paratuberculosis has been reported on every continent. Sweden and some states in Australia are the only regions of the world that claimed freedom from the disease over the last years. But recently there were reported findings of paratuberculosis in Sweden although with a very low prevalence (13).

The reported prevalence of infected animals varies by country and region and reaches up to 84.7 % MAP positive dairy herds in parts of Germany (14). 47.0 % positive dairy herds were found by Jakobsen et al. (15) in Denmark, van Leeuwen et al. (16) reported 43.0% positive dairy herds in Canada, and in Wisconsin, USA 50.0 % of the dairy herds were found to be positive for MAP (17). In Austria two representative studies were performed in the years 1994-97 and 2002-03 which showed, that the seroprevalence of antibodies against MAP increased from 6.97 % positive cattle herds in 1994-97 to 19.05 % positive herds in 2002-03 (18).

Pathogenesis

Most cattle with paratuberculosis are infected as young calves. Approximately 25 % of calves born to cattle with clinical signs of paratuberculosis are already infected in utero. Although calves are most susceptible, older heifers and adult cattle can become infected too (19). The primary site of MAP infection is the ileum, the most distal part of the small intestine. Specialized absorptive mucosal cells (M-cells) overlying small bulges of lymphoid cells near PEYER´s patches ingest MAP and discharge the organism into the subepithelial dome. Macrophages phagocytes the discharged bacillus from the M-cells and migrate into local lymphatics, spreading the infection to regional lymph nodes (10).

Transmission

The postnatal faecal-oral transmission is the most important means of exposure. After a long incubation period of up to 10 years (10) infected animals shed high quantities of MAP in their faeces. So called “super-shedders” can shed more than 1 million colony forming units (cfu) of MAP per gram of manure (which is more than 20.000 low shedding animals) without showing clinical signs (20). Although shedding of MAP is considered not to start before 1.5 years of age, recent studies showed, that also young calves can shed MAP and thereby pass infection to other calves in the same environment (21, 22). In newborn calves the infection mostly takes place soon after birth by oral ingestion of the organism. The most likely sources are faecal contamination of the calving unit as well as of the udder (if the calf is permitted to suckle). MAP can also be found in colostrum and milk of asymptomatic cows, serving as source of infection. Pasteurizing colostrum and milk is decreasing, but not avoiding this route of infection (19). Transmission via semen, by embryotransfer and from wildlife ruminant reservoirs has also been discussed (8, 23, 24). The vast majority of herds that acquire MAP do so through purchase of infected animals.

Clinical manifestations

Paratuberculois spreads slowly and it may be years before a herd is recognized as infected. Herds with good management practice may have a lower rate of infection than herds with poor management. Animal losses due to premature death within a herd can be high. Most clinical

cases occur between 3-6 years of age. Young infected cattle do not show clinical signs (silent infection). They seem identical to uninfected herdmates. No examination or tests are available to detect the infection at this stage, but as mentioned before, animals may shed infectious organism into the farm environment.

Subclinically infected adult animals do not show visible signs of paratuberculosis, although they carry MAP. In some cases specific antibodies to MAP and/or positive faecal culture results may be detected. However, most animals subclinically infected with MAP are culled for reasons unrelated to paratuberculosis.

The first clinical sign can usually be seen after calving (10) as a chronic or intermittent diarrhea alternating with periods of normal consistency of faeces. Associated with the diarrhea there is a generalized unthriftiness, a rough hair coat, dry skin and chronic weight loss despite normal or even increased appetite. Most animals test positive on faecal culture and show increased antibody titers at this time.

In advanced clinical disease affected cattle become weak and emaciated. Bottle jaw (intermittent edematous swelling between the rami of the mandible) typifies the disease. Decreased milk production, pipestream diarrhea and cachexia characterize the terminal stage of the disease. In sheep and goats diarrhea is usually absent. The prognosis is poor, the disease ends with the death of the affected animal. Up to now treatment strategies have not been successful. There are no drugs approved for the treatment of paratuberculosis.

Necropsy findings

In cattle the lesions found at necropsy are characterized by a diffuse granulomatous change, enteritis, without necrosis or reactionary fibrosis. The diseased animal is emaciated, shows serous atrophy of fat deposits, intermandibular edema and serous effusion into body cavitities. Lesions are limited to the gastrointestinal tract and regional lymph nodes. The intestinal mucosa, especially of the ileum, is visibly thickened and shows broad, transverse ("brain-like") folds. This pathognomonic corrugated appearance does not disappear when the intestine is stretched.

Economic significance

Paratuberculosis has emerged as one of the most prevalent and costly diseases of dairy cattle today. It also affects the beef cattle industry, particularly breeders of purebred cattle. While emacation and death are the final consequences of clinical paratuberculosis, infected cattle also suffer from decreased productivity associated with subclinical disease, infertility, mastitis and increased susceptibility to other diseases. The economic losses include decreased milk production, reduced value at slaughter, increased veterinary treatment costs, costs of disease control programs, as well as loss due to un- or underused production facilities. Indirect costs consists of export testing of live cattle, sheep and goats, testing for interstate movement of animals and funding for paratuberculosis research. Accurate estimates of the overall economic impact of the disease have not been made, because farmers prefer not to acknowledge its presence and enshroud suspect cases with secrecy. Thus it is difficult to ascertain the true prevalence or to estimate financial loss. Whitlock (19) estimated the total loss to exceed $ 1.5 billion annually for the dairy industry in the USA.

Diagnosis

Clinical cases of paratuberculosis can often be diagnosed based on clinical examination, history and necropsy findings. If clinical symptoms are missing laboratory tests have to be used to confirm or rule out the diagnosis. Unfortunately there exists no test for paratuberculosis with a high sensitivity and specificity, especially in young and subclinical infected animals. Many different test systems are available today for the detection of MAP, such as Ziehl-Neelsen Staining, faecal culture and Polymerase Chain Reaction (PCR) or the evaluation of antibody levels by Enzyme Linked Immuno Sorbent Essays (ELISA). Other methods like Complement Fixation Test, Interferon Gamma Assay, Gel Precipitation Test and Johnin Test which were used in the past are only used for research purposes today.

Microscopic detection of MAP in faeces by Ziehl-Neelsen Stain is a cheap and easy way to confirm diagnosis in clinical cases of paratuberculosis (25) which can be performed in practice. Unfortunately this method has a low sensitivity for the detection of subclinical infected animals (26).

Bacterial Culture from faecal or tissue (intestine, intestinal or liver lymph nodes) samples is one of the most widely used tests for the detection of MAP and serves as a gold standard. Different culture media and decontamination procedures are used to reduce contamination by other

microorganisms and increase culture outcome. Inkubation time for bacterial culture is 8-16 weeks (27) but can be reduced to 23 days by the use of liquid culture systems (28). Specificity of faecal and tissue samples is very high but sensitivity is low, due to the late onset of faecal shedding, intermittend faecal shedding and late occurrence of MAP in regional lymph nodes. To reduce costs, pooled faecal samples from up to 5 cows of the same age can be used in bacterial culture with a reported specificity of 86 % and sensitivity of 96 % compared to individual sampling (29).

PCR can be used to confirm the results of bacteriological culture as wall as to detect the specific insertion sequence IS*900* in faeces, tissue samples, blood and milk. Advantages of PCR are the high specificity of about 90 % and the possibility to gain results in less than 24 hours. Handicaps of the PCR are a low sensitivity of about 53 % (27) and that there is no possibility to differentiate between viable and non-viable MAP cells (27). Several ready-to-use PCR-kits are available today and many research groups and companies are currently working to improve this method.

ELISA for detection of specific antibodies against MAP is probably the most widely used screening test today (27). It is regularly used for blood (serum) samples but can also be used for milk (30). ELISA is easy and quick to perform and automation of the analysis is possible. Reported values for specificity and sensitivity vary between the different test- kits. Collins et al. (31) found a specificity of 84.7 % - 99.8 % and sensitivity of 27.8 – 44.5 % for five different ELISA. One mayor disadvantage of ELISA tests is the late humoral response in animals infected with MAP, leading to a late increase of antibodies and to negative results in young or recently infected animals (10). Nevertheless ELISA is one of the cheapest and most appropriate methods for herd investigations and MAP-control programs. If used for the diagnosis in single animals interpretation of results has to be critical and the test has to be redone, if necessary.

Although diagnostic tools for detection of paratuberculosis have made great progress and current research activities are high, there are still more questions than answers regarding the correct diagnosis, especially in young animals and animals in an early stage of infection. The selection of the right diagnostic tool, the combination of more than one test and reinvestigation of animals and herds are the most important keys to succeed in the diagnosis of paratuberculosis.

Control and prevention

Paratuberculosis is difficult to diagnose and untreatable. Therefore control and reduction in MAP positive herds and prevention of spreading the disease to negative herds is very important.

MAP positive animals should be culled from the herd as soon as possible and the whole herd has to be retested several times by combination of different diagnostic tests. At the beginning of a test and cull program all animals older than one and a half year should be tested every six month by ELISA and bacterial culture or PCR of faeces. To reduce costs, pooled faecal samples from up to five cows can be used (29). Animals shedding MAP should be removed from the herd immediately, serological positive individuals as soon as possible. For a quick decrease of the infection additional removal of all offspring from positive tested individuals should be performed. As soon as no more MAP shedding animals are found in a herd, test interval can be extended to once a year with the alternative use of ELISA and bacterial culture (PCR).

Additional hygienic precautionary measures have to be taken at positive farms to prevent further spreading of the disease in the herd. The mayor aim of the hygienic precautions is to prevent infection of newborns and young stock and to purchase MAP- free animals only. Optimal hygiene for parturition and immediate separation of neonates from their mothers as well as separation of offspring from antigen and antibody positive and negative mothers are the most important measures to prevent infection of new born animals. Furthermore only colostrum from antigen and antibody free individuals should be fed and only progeny from negative individuals should be used for breeding.

Young livestock has to be separated from adults on pasture for the first two years of the eradication program. To prevent spreading of MAP with feed and water, only plough –land should be fertilised with manure and pollution of water sources with manure has to be avoided. Shaded pastures should not be used for young animals and deer has to be kept away from pastures. The use of separate working equipment for young and sub adult animals and general improvement of cleaning and disinfection should also be performed. Detailed instructions and suggestions are given by Khol et al. (32).

Up to now there do not exist legal restrictions concerning paratuberculosis in livestock trade in Europe. A “European- standstill” for livestock trading and intensive herd testing would help to identify MAP-free regions and to prevent the fur-

ther increase of paratuberculosis in Europe. Furthermore only animals older than 1.5 years, tested negative for paratuberculosis or animals originating from negative tested herds should be introduced to MAP- free areas and herds.

Austria is the first country who will declare clinical paratuberculosis a notifiable disease followed by legally compulsory culling of clinical diseased animals and hygienic directions. This should help to maintain the low MAP prevalence in Austria and to remove MAP shedding animas from the herds (16)

Conclusion

Paratuberculosis or Johne´s disease is one of the most important diseases in ruminants today. Because of its long incubation period, high economic losses, difficulties in early diagnosis and discussed possible links to Morbus Crohn in humans, paratuberculosis will stay at the top of important diseases for veterinarians in the future. Greatly increased activities are needed to gain more knowledge about the disease and to develop reliable diagnostic tools in young animals.

Austria is the first country which is taking legal actions to prevent further increase of paratuberculosis in livestock but success is only possible if other countries follow and the disease is fought together on a European level.

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PARATUBERKULOZA (JOHNEJEVA BOLEZEN) PRI PREŽVEKOVALCIH - ZGODBA SE NADALJUJE

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Povzetek: Paratuberkuloza ali Johnejeva bolezen je danes ena najpomembnejših bolezni pri prežvekovalcih. Povzročitelj bolezni je Mycobacterium avium subsp. paratuberculosis (MAP), ki gostuje pri številnih živalih. Čeprav se bolezen običajno povezuje z govedom in drobnico, so zanjo dovzetni vsi prežvekovalci, vključno z divjimi in eksotičnimi vrstami le-teh. Delež okuženih živali je različen v različnih državah in pokrajinah, na nekaterih področjih je na povzročitelja MAP pozitivnih celo 84,7 % molznih čred. Za paratuberkulozo so najbolj občutljiva teleta, vendar se lahko okužijo tudi telice in odraslo govedo. Novorojenci se običajno okužijo takoj po rojstvu z zaužitjem povzročitelja iz okolice, MAP pa je bil dokazan tudi v mlezivu in mleku asimptomatsko okuženih krav. Čeprav običajno navajajo, da živali ne izločajo povzročitelja pred starostjo leta in pol, je bilo dokazano, da ga lahko izločajo tudi teleta in tako prenašajo okužbo na druga teleta v istem okolju. Velika večina čred se okuži prek nakupa okuženih živali. Prvi klinični znaki so običajno vidni po telitvi kot kronične ali intermitentne driske z obdobji normalne konsistence blata. Pri drobnici driske običajno ni. Subklinično okužene odrasle živali nimajo vidnih znakov okužbe, čeprav so nosilci MAP.

Prognoza je slaba, saj bolezen povzroča hujšanje in izčrpavanje živali, konča pa se s smrtjo. Če ni kliničnih znakov, je treba diagnozo potrditi ali ovreči z laboratorijskimi testi. Za ugotavljanje MAP so na voljo različni testni sistemi, kot so barvanje po Ziehl-Neelsenu, kultura blata in verižna reakcija s polimerazo (PCR) ali ugotavljanje ravni protiteles z metodo ELISA.

Ker je paratuberkulozo težko diagnosticirati in z MAP okužene črede nemogoče ozdraviti, so zelo pomembni preventivni ukrepi za preprečevanje širjenja povzročitelja na neokužene črede. Znotraj črede preprečujemo nadaljnje širjenje bolezni s higienskimi ukrepi, njihov glavni cilj je preprečevanje okužbe telet in mladih živali, pri nakupu pa je treba paziti, da so živali zdrave.

V članku je prikazan pregled načina okužbe, diagnostičnih postopkov in ukrepov za nadzor te bolezni. Osnove smo kombinirali z novimi vidiki sodobnih raziskav in s tem podali natančne in sodobne informacije o paratuberkulozi za veterinarje, ki se ukvarjajo s čredami molznih živali.

Ključne besede: govedo, bolezni; paratuberkuloza – diagnostika – epidemiologija; preprečevanje in nadzor; *Mycobacterium paratuberculosis* – patogeneza; govedo; ovce