

## PARATUBERCULOSIS IN CATTLE – THE OUTSPREAD, DIAGNOSTICS AND CONTROL

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Invited paper

**Abstract:** Paratuberculosis is chronic incurable granulomatose enteritis, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease can be found in cattle, sheep and goats and it can appear among all age groups. The disease spreads slowly, the condition can vary, but it always ends with severe dehydration, weight loss and complete exhaustion of the animal. Diagnosis of paratuberculosis is established by direct detection of causative agent using selective media or by detection of agents' genome using the PCR method. Indirect methods are based on the detection of specific antibodies in blood sera or milk, or on the measuring of cellular immunity. The detection of antibodies using ELISA method is considered the method of choice for the diagnosis of paratuberculosis, because of the rapidity of the test and relatively low expenses. The first serological analysis on the presence of paratuberculosis in cattle was carried out 20 years ago in the region of AP Vojvodina (Republic of Serbia). Blood sera taken from cattle originating from 12 farms were examined. The AGID (agar gel immunodiffusion) test revealed positive results in 13 cows coming from four farms, which makes 1.5% from the total number of cows. Furthermore, CF (complement fixation test) method revealed 35 positive cows, which makes 4.1% out of the total number of cows. In 1991, similar analysis applying ELISA test revealed 29 positive cows, i.e. 2.9% of positive cattle, which reflects a favourable epizootiological situation in the region. Lack of well-established laboratory tests, long periods of incubation and small number of clinical cases impede appropriate control of paratuberculosis. Control programs are based on reduction of transmission of the agent to host animals, elimination of infected animals, hygiene-sanitary measures and vaccination. The efficacy of the recommended programs would directly depend on elimination of infected animals. Despite the continuous research and numerous studies, the problem of detecting the infection caused by MAP is still present. This fact, together with the complex procedure of laboratory diagnostic, has caused a permanent spreading of the infection in cattle herds, while measures taken so far for the control of paratuberculosis have not been efficient enough.

**Key words:** paratuberculosis, cows, diagnostics, control measures

## Introduction

Paratuberculosis is chronic granulomatose enteritis, caused by *Mycobacterium avium* subsp. *paratuberculosis*. The disease can appear in cattle, sheep, goats, in all age categories. Paratuberculosis has spread throughout many countries of Europe, USA, Australia, Canada, Japan, South America and even some African countries. According to the numerous studies, it has been found that number of infected animals has significantly increased (*Bannantine et al., 2002; Cousins et al., 1995; Kalis et al., 1999*). The disease has been widely spread in cattle in Europe. The mortality rate in infected herds is around 1% and in some cases even 10%. It is hard to determine the prevalence of the disease in a certain region, because diagnostic procedure is complex and not always reliable. In addition, the cases of disease incidence are not always reported, unless research is being done or eradication program is being applied. Despite the continuous research, the problem of detecting cases of subclinical infection is still present. Complex and time-consuming diagnostic procedure resulted in permanent spreading of the infection in ruminant herds, whilst control measures have not been efficient enough.

**Aetiology and pathogenesis.** Paratuberculosis is a disease caused by *Mycobacterium avium* subspecies paratuberculosis (MAP). It is a non-moveable gram-positive acid-resistant microorganism. In nutrition media, described by Smith (*Nielsen et al., 2004*), after 4-6 weeks of incubation at 37-39°C, MAP forms small, elevated, milky white, rough colonies with irregular margins. Three months later, the colonies grow up to 2mm, especially in the case when they coalesce. Morphology of the colonies depends on the features of used media. Also, a growth factor called mycobactin is necessary.

Animals can be infected by food and water contaminated with faeces from infected animals. The disease spreads by trading animals with latent infection. Infected animals, due to the long period of incubation, can excrete the causative agent for 15-18 months, before the appearance of clinical symptoms. MAP can be isolated from milk and colostrums of cows with subclinical and clinical signs of disease, which enables infection of calves. Calves are especially sensitive in the first few months of life when major source of infection is contaminated milk. In the environment, MAP is found to be sensitive to sunlight, drying, high level of calcium and high pH of the soil. Factors that support the appearance of clinical signs are infective dose, poor nutrition, as well as sudden changes in nutrition, age, stress, partus, transportation and immunosuppressive agents like bovine viral diarrhoea (BVD) virus.

MAP is one of the possible agents connected to Crohn's disease in humans, so milk and dairy products are considered possible source for infection in humans.

**Clinical symptoms.** Chronic diarrhoea and progressive weight loss are the most common clinical symptoms. In cattle, clinical symptoms do not appear before two years of age. Most frequently, they appear between the age of 2 and 6 years. The disease is in the beginning most often visible in one animal, and it spreads slowly. There is a drop of milk yield in cows before diarrhoea occurs. Animals' appetite is normal, same as the body temperature, while on the other side thirst increases. Diarrhoea can be intermittent or continuous. The course of the disease varies, but it always ends as a severe dehydration, weight loss and complete exhaustion of the animal. In infected herds, clinical form of the disease can be seen in only 3-5% of animals. In dairy cows with clinical symptoms, milk production decreases by 19.5% compared to their lactation two years before. Decease of milk production around 6-16% is also found in herds with subclinical infection (*Kalis et al., 1999*).

**Laboratory diagnostics.** Laboratory diagnosis of paratuberculosis is possible in animals with clinical symptoms, but also for detection of subclinical infection in animals. Two major goals of laboratory diagnostics are to analyze the presence of infection in the herd and to establish reliable diagnosis on the level of a single animal (*Vidić et al. 2010*).

*Direct detection of agent.* Bacteriological analysis of faeces is valuable diagnostic procedure for detecting cows with clinical symptoms and subclinical cases as well. This method is considered reliable for indication of infection in live animals even 1-3 years before the appearance of clinical symptoms.

a) Isolation from faeces (Faeces culturing)

Despite the fact that this method is time-consuming and laborious, it is still the most reliable method for the diagnosis of paratuberculosis (*Kalis et al., 1999*). Sensitivity of the method depends on disease stadium, while specificity is considered to be 100%. In routine diagnostic work, the most used method is isolation in solid media and lately liquid media has been in usage (*Nielsen et al., 2004*). Culturing in liquid media produces better results, culturing time is shortened, but the method itself is more complex. Isolation is impeded by sample contamination (other bacteria, moulds, fungi), secretion of a small number of bacteria, intermittent secretion, necessity of specific media and slow growth (3-5 months). MAP demands for its growth addition of mycobactin, which serves for phenotype characterization of MAP colonies. This supplement shortens time until first colonies appear from 13 to 3 weeks (*Harris et al., 2005*).

b) Polymerase chain reaction (PCR)

Different sequences have been identified for the molecular identification of MAP (*Möbius et al. 2008*). Most frequently, the sequence is IS900, but similar sequences were found in other bacteria too. That is why only specific IS900 pairs of primers are recommended for use. During the last few years, other specific sequences have been detected, such as f57, lokus255, ISMap02 and other (*Stabel and Bannantine, 2005*). So far, the sensitivity of PCR method has been limited by

the efficacy of DNA extraction. Level of detection by PCR depends on the amount of agent in faecal samples and ranges from 80-100% in samples with great amounts of the agent.

*Indirect detection.*

a) Detection of specific antibodies against MAP

In advanced cases of infection, specific antibodies appear as a reaction of immunologic response. Sensitivity of serologic diagnostic methods for paratuberculosis is lower compared to other infections. Different data about sensitivity of methods can be found in literature - from 6.9% to 88.2%. It varies upon the antigen used, structure of the animal population tested and golden standard chosen for the characterisation of infected and noninfected animals (*Robbe-Austerman et al., 2006*). Detection of antibodies with ELISA method is considered as a method of choice for diagnostic of paratuberculosis, because it is rapid, cheap and it can be used in control programs for paratuberculosis (*Grgić et al., 2008*). Specific antibodies can be detected in milk and blood samples, whilst sensitivity of ELISA test depends on form of disease in infected animals (*Dargatz et al., 2001; van Weering et al., 2007*).

Agar gel immunodiffusion test (AGID) and complement fixation test (CF) were used previously as traditional methods, but their usage is ceasing (*Vidić et al., 2002*).

b) Detection of cellular immunity

After per os infection in first few months of life, the causative agent penetrates the mucosa of small intestine and goes into lymphoid system via M cells. Macrophages induce cellular immunity after phagocytising of bacteria. MAP can survive and replicate inside macrophage and activated macrophage begins activation of T cells and production of characteristic cytokines. Production of gamma interferon is the earliest detectable reaction in MAP infection (*Kalis et al., 2003*).

For the purposes of diagnostic of paratuberculosis, allergic test can be used, as well. However, this test is not widely used any more because of insufficient sensitivity and specificity. Other methods for detection of infected animals described in literature are lymphocyte transformation test, inhibition of leukocyte migration and gamma interferon test (*Huda et al., 2003*).

In the epizootiological region of southern Bačka and Srem, a previous study on paratuberculosis was done 20 years ago (*Vidić et al., 2001*). Representative number of blood samples from cattle were analysed from the region of southern Bačka and Srem. In total, 845 blood sera from 12 farms were analysed. For the detection of specific antibodies against MAP, two methods were used: AGID test and CF test. The conclusion of this study was that infection of paratuberculosis is present and maintaining in the observed region. The AGID test revealed positive results in 13 animals from 4 farms, (1.5%), while CF test revealed 35 positive animals (4.1%). It is evident that larger number of animals and larger number of herds positive for paratuberculosis were found using CF method. Titres

in positive samples ranged from 1:8 to 1:64, while GMT was 18.3 (*Vidić et al., 2001*).

## Materials and Methods

The investigation was carried out in South Backa and Srem district. Freely chosen representative sample of the cattle from all municipalities of this areas were included in examination. The cattle derived from the farms where the number of animals ranged from 5-300, as well as from private households. The cows tested for paratuberculosis were older than two years and were the Holstein-Frisian and Simmental breed and indigenous colourful breed. The samples were submitted by the veterinary service on the filed.

The examination included 1000 cow serum samples. Indirect ELISA (HerdChek IDEHH Lab.) was used for detecting specific antibodies against *M. paratuberculosis*. The sample to positive ratio of the samples is calculated by using the absorbance (A450 or A450/620) obtained with the test sample and a positive control (S/P), corrected for the absorbance of the negative control. Bovine samples with S/P ratios of less than or equal to 0.15 are classified as negative for *M. paratuberculosis* antibodies, samples with S/P ratio between 0.15 and 0.30 are classified as suspicious or doubtful, and samples with S/P ratio of greater than or equal to 0.30 are classified as positive.

## Results and Discussion

The results obtained from the study are presented in Tables 1 and 2. The ELISA test applied to blood sera of cattle revealed paratuberculosis positive results in 29 animals (2.9%).

**Table 1. Results of ELISA test of blood serum samples on presence of paratuberculosis in the cattle originating from south Bačka region**

Municipality	No. cows	% of analysed	No. of analysed	doubtful		positive	
				No.	%	No.	%
Bač	1068	2.60	26	-	-	-	-
Bačka Palanka	3601	2.78	88	5	5.68	-	-
Bački Petrovac	503	1.22	12	1	8.33	1	8.33
Bečež	5031	12.27	123	7	5.69	6	4.87
Novi Sad	2955	7.20	72	3	4.16	2	2.77
Srbobran	850	2.07	20	-	-	1	5.00
Temerin	1063	2.59	25	2	8.00	-	-
Titel	3710	9.04	90	1	1.11	2	2.22
Žabalj	3475	8.47	84	4	5.95	5	5.95
Total	22256	54.28%	540	22	4.07	17	3.14

**Table 2. Results of ELISA test of blood sera samples on presence of paratuberculosis in cattle originating from Srem region**

Municipality	No. cows	% of analysed	No of analysed	doubtful		positive	
				No.	%	No.	%
Beočin	889	2.16	21	4	19.04	-	-
Indija	3069	7.48	75	4	5.33	1	1.33
Irig	1268	3.09	30	2	6.66	2	6.66
Pećinci	2851	6.95	69	3	4.34	3	4.34
Ruma	2549	6.21	62	-	-	-	-
Sr. Karlovici	87	0.21	3	-	-	-	-
Sr. Mitrovica	3577	8.72	87	2	2.29	2	2.29
St. Pazova	3013	7.34	73	-	-	2	2.73
Šid	1668	4.06	40	5	12.5	2	5.00
Total	18971	46.27%	460	20	4.34	12	2.60

Based upon the obtained results, it can be concluded that the level of seroprevalence in the observed region is actually low, compared to the data published from other countries (*Cousins et al., 1995; Kalis et al., 1999; Nielsen et al., 2004*). However, the obtained results do not reflect the extent of the MAP infection. Low level of infected cows would be recommended to maintain by the application of certain measures, which would prevent the importation of paratuberculosis infection into our herds. This applies to mini farms or larger farms of dairy cows and to newly formed herds of cows.

Until now, the efficient therapy for paratuberculosis still has not been found. Vaccination can enforce elimination of clinical form of the disease and reduce spreading of the infection. Lack of good laboratory tests, long period of incubation, as well as small number of clinical cases, make the control of paratuberculosis very difficult. Control program has been based on reduction of agent transmission to susceptible animals, elimination of infected ones, vaccination and measures of hygiene. Conservative method of eradication has been based on identification of agent „excretors“ by serologic methods and also slaughtering of animals with clinical symptoms. After continuous research about paratuberculosis, the problem of detecting the infection is still present. Together with a complex procedure of laboratory diagnostics, it has caused permanent spreading of the infection in cattle herds meaning that the control measures for paratuberculosis were not efficient enough. A precise data on presence and prevalence of paratuberculosis are necessary for the beginning of the control program, but gaining those data is limited because of the insufficiency of a suitable „screening“ test for detecting the subclinical infection.

Numerous researchers are investigating novel procedures for the diagnostics of paratuberculosis or are trying to improve the existing ones (*Bannantine et al., 2002; Cousins et al., 1995; de Juan et al., 2006*). There is a

need for a test, which could detect infection in young animals and establish accurate diagnosis on the level of individual animals. However, the control of paratuberculosis is possible even now with all the problems still present if the regular diagnostic is combined with strict sanitary measures and removal of infected animals.

## Conclusion

Paratuberculosis was detected in the cows in the South Backa and Srem district. ELISA test was used in testing cattle blood serum and 29 positive results were detected, i.e. 2.9% cattle were paratuberculosis-positive. If the result were expressed as the total number of cattle that are positive and suspect to paratuberculosis, it would make 5.3% of 1000 tested cattle serum samples.

Based on the obtained results it can be evaluated that the level of seroprevalence is relatively low and that the infection has not spread in the last 20 years. This level of prevalence of paratuberculosis is encouraging. Implementation of appropriate preventive measures is important to maintain this level, i.e. to prevent the spread of the infection in the herds.

## Acknowledgment

Research was financed by the Ministry of Education and Science, Republic of Serbia, project 20133 Control of biohazard agents in farms and implementation of biotechnological procedures for reduction of risk in safe food system and safe disposal of waste.

## Paratuberkuloza kod goveda – raširenost, dijagnostika i kontrola

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## Rezime

Paratuberkuloza je hronični neizlečivi granulomatozni enteritis uzrokovan sa *Mycobacterium avium* subsp. *paratuberculosis*. Bolest se javlja kod goveda, ovaca i koza, a zahvata sve starosne kategorije. Bolest se širi lagano, tok bolesti varira, ali se uvek završava kao ozbiljna dehidracija, mršavljenje i potpuna iscrpljenost životinje. Postavljanje dijagnoze paratuberkuloze vrši se direktnim dokazivanjem uzročnika primenom selektivnih podloga ili dokazivanjem genoma

agensa PCR metodom. Indirektni metodi podrazumevaju utvrđivanje specifičnih antitela u krvnom serumu i mleku ili merenje ćeljskog imuniteta. Dokazivanje antitela ELISA testom smatra se metodom izbora za dijagnostiku paratuberkuloze zbog brzine izvođenja i relativno niske cene koštanja. Prva serološka ispitivanja prisustva paratuberkuloze u goveda vršena su pre 20 godina na području AP Vojvodine. Ispitivanjem su obuhvaćeni krvni serumi krava sa 12 farmi. Primenom AGID (agar gel immunodiffusion) testa pozitivni nalazi dobijeni su kod krava sa četiri farme, odnosno kod 13 životinja ili 1,5%. Metodom RVK utvrđeno je 35 serološki pozitivnih krava ili 4,1%. U ispitivanjima nakon 15 godina primenom ELISA testa ustanovili smo 29 pozitivnih seruma, ili 2,9% pozitivnih goveda, što govori u prilog povoljne epizootiološke situacije.

Nedostatak dovoljno meritornih laboratorijskih testova, dug period inkubacije i mali broj kliničkih slučajeva otežava kontrolu paratuberkuloze. Programi kontrole baziraju se na smanjenju transmisije agensa na prijemljive životinje, eliminacija inficiranih životinja, mere higijene i vakcinaciju. Efikasnost preporučenih programa zavisila je direktno od eliminacije inficiranih životinja. I pored kontinuiranih i mnogobrojnih istraživanja problem otkrivanja infekcija izazvanih sa MAP je i dalje prisutan. Ta činjenica, kao i složen postupak postavljanja laboratorijske dijagnoze, usloveli su permanentno širenje infekcije u zapatima preživara, stoga preduzete mere u kontroli paratuberkuloze nisu bile dovoljno efikasne.

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Received 30 June 2011; accepted for publication 15 August 2011