

Occurrence of faecal shedding of *Mycobacterium avium* subs. *paratuberculosis* by calves^{*)}

AGNIESZKA WISZNIEWSKA-ŁASZCZYCH, JOANNA SZTEYN, KATARZYNA LIEDTKE, BEATA WYSOK, JOANNA WOJTACKA, MAŁGORZATA GOMÓŁKA-PAWLICKA

Department of Veterinary Protection of Public Health, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 14, 10-719 Olsztyn, Poland

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Summary

Paratuberculosis or Johne's disease is a chronic granulomatous enteric disease afflicting cattle, sheep, goats and other ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Calves are infected mainly via an oral route, especially from manure and environment contamination by infected adult cattle. It is generally assumed that calves rarely shed MAP bacteria and that calf-to-calf transmission is of minor importance. The aim of the study was to evaluate occurrence of faecal excretion of MAP by calves born to MAP-positive and MAP-negative dams in a low MAP prevalence herd.

The study involved 66 dam-calf pairs. Samples of faeces were collected: for each cow once about 2-3 weeks to parturition, and for each calf 3 times at 2-5, 180-185 and 360-365 days of age. All samples were studied for the presence of MAP by culture or by PCR of IS900 insertion sequence MAP DNA. Faecal samples of the dams demonstrated 15/66 dams with at least one positive result. Analysis of MAP shedding prevalence in the faeces of calves originating from MAP-positive and MAP-negative dams shows that in 15 MAP-positive dams: 10 out of 15 calves (66.66%) at the age of 180-185 days and at the age of 360-365 days 11 out of 15 calves (73.33%) were MAP-positive in faecal samples. In the case of MAP-negative dams: 7 out of 51 calves (13.72%) at the age of 180-185 days, and 8 out of 51 calves (15.68%) at the age of 360-365 days were MAP-positive. MAP-infection cases in calves coming from infected and healthy dams prove the occurrence of calf-to-calf transmission and that contact with infected calves increases the risk of MAP spreading among healthy calves.

Keywords: *Mycobacterium paratuberculosis*, calves, MAP-shedding

Paratuberculosis, also known as Johne's disease (JD), is a chronic granulomatous enteric disease afflicting cattle, sheep, goats and other ruminants, both domestic and wild. The disease is caused by the *Mycobacterium avium* subspecies *paratuberculosis* (MAP), an acid-resistant, slow-growing microorganism of the *Mycobacterium avium* complex. Infection usually takes place in young animals (≤ 6 months of age) (2, 15, 39), and the clinical symptoms of the disease usually appear in older animals (4, 12, 20, 33).

The full clinical picture of the disease appears only in a low number of cows in a herd (19). Paratuberculosis occurs much more frequently in a subclinical form, whose course comprises two stages: first, in which the infected cows do not excrete mycobacteria, and the second, in which shedding of mycobacteria is present

(21). Mycobacteria are excreted with faeces, colostrum and milk, by both animals with symptoms of JD and by those infected but without disease symptoms.

Diagnosis of the disease is difficult owing to its long period of incubation (4, 9) and depending on the severity of clinical symptoms, potential excretion of the mycobacterium to the environment, and the possibility of detecting the disease with contemporarily known methods.

Calves acquire the infection mainly through the oral route, especially from manure and environmental contamination by infected adult cattle (32). An important infection factor is also colostrum and milk of infected animal origin. Calves born to MAP positive dams have a higher infection risk due to the possibility of in-utero transmission and due to contact with infectious faeces and uptake of infectious colostrum at parturition. (25, 36). Oral inoculation of calves has generally resulted in a subclinical form of the disease and very rarely resulted in a clinical form. It was generally assumed

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that MAP infected calves are not infectious until ≥ 2 years of age (4). This is why almost all models for paratuberculosis control programs at the herd level are based on prevention of dam-to-calf transmission by avoiding calves' exposure to adult cattle manure, raw colostrum and milk (3, 8, 37). It was thought that segregating young stock from adult cattle may be sufficient to stop MAP transmission in an infected herd (3, 8, 25). Experimental studies have suggested that calves and young animals (< 2 years of age) could excrete MAP in their faeces, and MAP can be transmitted horizontally in young stock groups (calf-to-calf transmission) (25, 26, 35). It has been reported that the clinical form of JD appears in heifers ≤ 2 years old (42). Faecal shedding of MAP by calves may play a role in transmission of paratuberculosis infection in calves housed together in groups (38) and play a role in the maintenance of paratuberculosis within herds.

Limited studies have been undertaken to determine the MAP infection status of calves originated from naturally infected herds. The aim of the study was:

- evaluation of the occurrence of faecal excretion of MAP by calves born to MAP-positive and MAP-negative dams in a low MAP prevalence herd;
- defining whether the standard handling procedures in herds infected with MAP protect against transmission of paratuberculosis infection in calves.

Material and methods

Herd selection. The study was conducted in a single commercial dairy herd with endemic presence of JD and single maternity pens, located in the Żuławy Wiślane region (northern Poland). The number of cattle in the selected herd was 150 at the time of sampling. The within-herd prevalence for MAP infection determined on the basis of the ELISA test (Institut Pourquier, France) was 6.5%.

New-born calves were separated from their dams and were kept separately for 30 days. After one month of life, they were joined together into one young stock group. All calves, regardless their origin from MAP-seropositive or MAP-seronegative dams, were fed by colostrum obtained from MAP-seronegative dams and milk replacement formulas.

Sample collection. The study involved 66 dam-calf pairs. For each cow, about 2-3 weeks to parturition, samples of faeces via the rectum were collected by a veterinarian. For each calf, a faecal sample via the rectum was collected by a veterinarian 3 times at 2-5, 180-185 and 360-365 days of age. Faecal samples in sterile containers was labelled with the identification of the dam and the calf. All samples transported within 6 hours to the laboratory for analysis.

Laboratory analysis. All faecal samples were evaluated for the presence of MAP by direct detection of MAP DNA by PCR and the classical culture method.

For direct detection of MAP DNA, 5 g of faeces was dissolved in 10 ml of sterile water and gently mixed for 30 minutes. After that, the sample was left for a further 30 minutes for sedimentation. 200 μ l of supernatant was subjected to a reaction with the Genomic mini kit (A&A

Biotechnology, Poland) to extract DNA. The genetic material was subjected to the PCR. To carry out the reaction, a mixture was prepared with: 25 μ l of PCR Master Mix Rapid (A&A Biotechnology, Poland); 0.5 μ l of 100 pmol primer P90+ (5'-GAAGGGTGTTCGGGGCCGTCGCT-TAGG-3'); 0.5 μ l of 100 pmol primer P91+ (5'-GGCGTT-GAGGTCGATCGCCACGTGAC-3') (16); and 14 μ l of water free of DNases. The reaction mixture (45 μ l) was mixed with 5 μ l of the examined material, transferred to a thermocycler (Mastercycler – Eppendorf) and amplified. The course of the reaction was as follows: denaturation for 15 min at a temperature of 94°C; 30 cycles – 1 min at 94°C, 45 seconds at 67°C and 2 min at 72°C; termination of the reaction – 2 min at 72°C. The resultant amplification product was stored at a temperature of 4°C until subjected to electrophoresis, which was conducted in 1.5% LSI agarose gel, in a TAE buffer (0.4 g LSI Agarose ME + 25 ml 1 $\times$ TAE buffer). Next, 8 μ l of the PCR product was mixed with 2 μ l of the loading buffer, injected onto the gel and subjected to electrophoresis at 100 V for 60 min. in the presence of a 1 kb ladder marker (Gibco).

The classical culture method has been described previously by Whitlock et al. (40). The pellets derived from each faecal sample were dissolved in 1 ml of a solution of amphotericin B in sterile water (0.0001 g/ml) and plated onto two Herrold's Egg Yolk Agar Slants (HEYM) enriched with Mycobactin J. Inoculated slants were incubated in 37°C. 8 weeks post inoculation, the HEYM slants were examined for growth of MAP. Slants without visible growth were examined weekly for the next 8 weeks. Colonies were confirmed by PCR for belonging to MAP genus. Samples were reported as positive or negative when at least on one slant with MAP's growth was confirmed.

Statistical analyses. Data was analysed statistically using Statistica 9.0PL software by Pearson Chi square test.

Results and discussion

Evaluation of the presence of MAP in faecal samples of the dams by the cultivation method and direct isolation of DNA-MAP demonstrated 15/66 dams with at least one positive result that was classified as MAP positive. 12 faecal samples were determined positive both for cultivation and direct detection of MAP DNA, while 2 positive only for direct detection of MAP DNA and 1 positive only for the cultivation method.

Faecal samples of calves at the age of 2-5 days have not demonstrated positive results both by the cultivation method and direct detection of MAP DNA, regardless of the origin of calves from MAP-positive or MAP-negative dams. Analyses of faecal samples from 180-185 day-old calves demonstrated 17 animals (25.75%) with at least one MAP-positive result. At the age of 360-365 days, MAP-positive was demonstrated by 19 (28.78%) calves. All 180-185 day-old MAP-positive calves were also MAP-positive at the age of 360-365 days. In 3 cases, 180-185 day-old calves with positive results only in direct detection of MAP DNA from faecal samples were also MAP-positive in the cultivation method at the age of 360-365 days. In 3 cases,

180-185 day-old calves with positive results only in the cultivation method were also MAP-positive in direct detection of MAP DNA from faecal samples at the age of 360-365 days. Calves with at least one positive faecal sample result were classified as MAP-positive.

Analysis of MAP shedding prevalence in the faeces of calves originating from MAP-positive and MAP-negative dams shows that in 15 dams with at least one MAP-positive result for faecal samples, 10 calves (66.66%) at the age of 180-185 days had MAP-positive faecal samples. Calves aged 360-365 days from 11 infected dams (73.33%) were MAP-positive in faecal samples. In the case of MAP-negative results for faecal samples in dams, 7 out of 51 calves (13.72%) aged 180-185 days from such dams were MAP-positive in faecal samples and 8 out of 51 such calves (15.68%) were MAP-positive at the age of 360-365 days. Comparison of the results from calves of MAP-positive and MAP-negative dam origins indicates that there are considerable differences in both groups (Fig. 1). Further analyses by the Pearson Chi square test revealed that the difference between the two groups at the age of 180-185 days and 360-365 days was statistically significant ($p < 0.01$).

There are only a few works describing the development of the infection in a herd in field conditions. A thesis that the MAP infection status of the dams is a significant predictor of MAP infections in calves (1, 2, 43) is consistent to previous dam-calves pair studies. This thesis has been confirmed by research conducted by Bolton et al. (2), showing the possibility of infecting calves during the perinatal period and during shedding of MAP by calves born within infected dams. Bolton et al. (2) showed 8% of 0-90 day-old calves born to MAP-positive dams shed detectable levels of MAP in their faeces. In our research conducted in a herd with low prevalence, we detected shedding of MAP in faeces by calves aged 6 and 12 months. Moreover, we also demonstrated a statistically significant difference between the origins of calves born by MAP-positive dams and the frequency of the shedding of MAP with faeces. In the research conducted by us, we demonstrated that, similarly to suggestions by many other researchers, calves from MAP-positive dams shed MAP much more often compared to calves from MAP-negative dams. A majority of the calves subjected to our research were recognized in the research as MAP-positive, irrespective of their origins from MAP-positive or MAP-negative dams, shed MAP with faeces at the age of 6 months (180-185 days). The

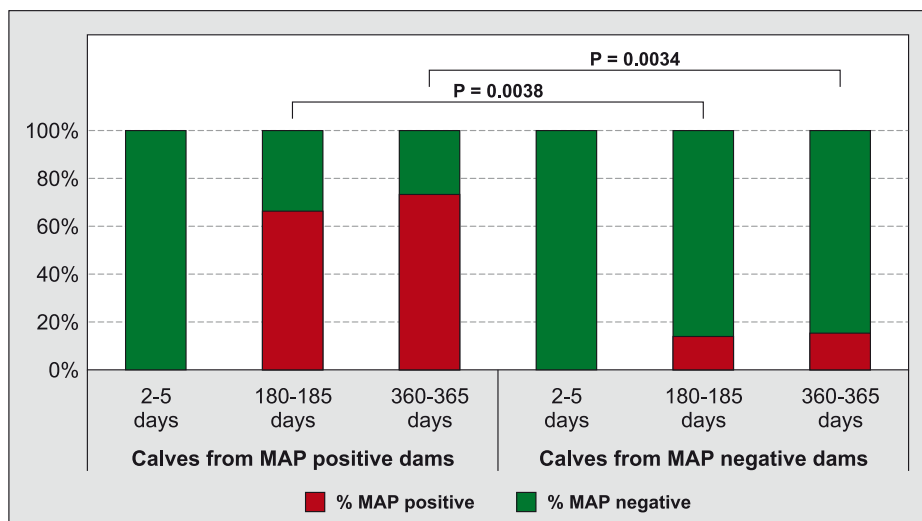


Fig. 1. Presence of MAP-positive and MAP-negative calves at different ages and dams status

Explanations: red bars represent MAP-positive calves and green bars represent MAP-negative calves; P – statistical significance between calves groups originating from MAP-positive and MAP-negative dams

total number of calves shedding MAP at the age of 12 months grew only slightly compared to the number of calves shedding MAP at the age of 6 months. In addition, it has been noticed that all calves shedding MAP with faeces at the age of 6 months also shed MAP at the age of 12 months.

Literature provides descriptions of numerous experiments showing the development of infection and shedding in calves experimentally infected with various MAP doses. This research indicates the dependency between the infectious dose and the age of calves and the frequency and time of occurrence of shedding. Mortier et al. (18) noticed that calves infected before the 6th month of age demonstrate shedding more often compared to those inoculated at an older age. He also noticed the fact that calves inoculated with a large MAP dose (5×10^9) demonstrated MAP shedding more often than calves inoculated with a low MAP dose (5×10^7), and this correlation disappeared when animals older than 6 months were infected. Moreover, in experimental models, it was shown that calves inoculated with a high dose of MAP at a younger age (below the 6th month of life), the clinical form of Johne's disease developed faster (25, 36, 41).

Meta-analysis of MAP experiments concluded that the average time to first shedding was 3 months, and most shedding was detected within 6 months after inoculation (17). Stabel et al. (30) and Subharat et al. (31) achieved similar results when they observed initial cases of MAP shedding in the faeces of calves 2-4 months from oral inoculation. In addition, similar shedding occurrence timing was observed among others in experimentally infected goats (7), sheep (9) and even in the mouse-model, where the highest shedding peak occurred 4 months after infection (6).

Research conducted by Pithua et al. (23) shows contrary results; in calves originating from a herd with

a high prevalence of MAP, no MAP shedding was observed in calves up to 90 days old. However, this research comprised a small number of dam-calf pairs, and only 8% of dams were faecal culture positive for MAP. The spread of the infection under field conditions remains unknown, together with the infection dose, which is impossible to define; and consequently, the time, occurrence or lack of and age of infection in calves is difficult to determine. The results of experimental infections show that the time from the moment of infection up to the occurrence of shedding in faecal samples amounts to at least 2 months, and only after 6 months do all infected animals shed MAP. Moreover, it was proven that MAP shedding in faeces may be occasional or continuous both in the case of clinical signs of the disease and without them (2, 18, 25). Infected calves in the subclinical stage of Johne's disease may shed MAP at low levels (14), and some shedding might have been missed, especially considering that the sensitivity of a faecal culture is relatively low and estimated at 23-74% depending on the "gold standard" method (19). Moreover, based on previously conducted observations, we know that the probability of infection is much higher in herds where clinical cases of Johne's disease occur regularly compared to herds where only subclinical cases are observed. In addition, more frequent shedding was observed in herds with high prevalence compared to herds with low prevalence (36).

Comparing the results achieved in our work with results achieved in experimentally infected calves, we may suppose that the calves examined by us were infected during the first months of their lives. The calves were soon separated from their dams, and all of them, irrespective of whether they originated from MAP-positive or MAP-negative dams, were initially fed with colostrum and later with milk obtained from seronegative cows up to the moment when they were fed with solid feed. Consequently, a probable infection source was calf-to-calf transmission. Calf to calf infection was also proved by Corbett et al. (5) in tissue culture of experimentally infected calves.

There is a possibility of calves infection by a contaminated environment. Data reported by Krauze et al. (11) show that in Chilean dairy herds MAP was isolated from the samples collected from areas strongly contaminated by manure such as manure storage lagoons and holding pen floors (22% culture-positive samples) but there were no culture-positive samples collected from paths and housing areas. Studies conducted by Pithua et al (22) and MacAloon et al. (13) found no difference in passive transfer of MAP infection between calves born in single and group calving pens. That may prove the limited role of environmental contamination on calves infection, especially in herds of high and average hygiene level. Use of individual calving pens is still recommended in herds with endemic paratuberculosis to reduce possibility of calves infection

(21, 34). MAP contamination level of environment samples is correlated with number of shedding cows in the herd (28). Calves born in high prevalence herds are more likely be infected than calves originating from low prevalence herds. To limit herd seroprevalence Kirkeby et al. (10) proposed a new framework for simulating MAP infection within a herd, based on a statistical method. They observed a synergistic effect of implementing three most popular herd procedures: remove calves from cows within 2 h; feeding calves with colostrum and milk obtained from seronegative cows or pasteurize; cull repeatedly positive cows. It could help farmers improve MAP control programs.

MAP-infection cases in calves coming from infected and healthy dams prove the occurrence of calf-to-calf transmission and that contact with infected calves increases the risk of MAP spreading among healthy calves. Consequently, in order to restrict MAP infections in the herd, apart from controlling adult animals and separating calves from infected dams, a calf-to-calf transmission prevention programme should be introduced.

References

1. Aly S. S., Thurmond M. C.: Evaluation of *Mycobacterium avium* subsp. paratuberculosis infection of dairy cows attributable to infection status of the dam. *J. Am. Vet. Med. Assoc.* 2005, 227, 450-454.
2. Bolton M. W., Grooms D. L., Kaneene J. B.: Fecal shedding of *Mycobacterium avium* subsp. paratuberculosis in calves: Implication for infection control and management. *Proc. 8th Internat. Colloq. Paratuberculosis 2005*, 596, 600.
3. Caldwell G. L.: The prospects for herd level control of paratuberculosis in cattle: a consultant's view. *Proc. 8th Internat. Colloq. Paratuberculosis 2005*, 165, 173.
4. Chiodini R. J., Van Kruiningen H. J., Merkal R. S.: Ruminant paratuberculosis (Johne's disease): the current status and future prospects. *Cornell Vet.* 1984, 74, 218-262.
5. Corbett C., De Buck J., Orsel K., Berkema H.: Transmission of infection among group-housed calves inoculated with *Mycobacterium avium* subsp. Paratuberculosis. *Proc. 12th Internat. Colloq. Paratuberculosis 2016*, 04, 1.
6. Hamilton H. L., Cooley A. J., Adams J. L., Czuprynski C. J.: *Mycobacterium paratuberculosis* monoassociated nude mice as a paratuberculosis model. *Vet. Pathol.* 1991, 28, 146-155.
7. Hines M. E. 2nd, Stiver S., Giri D., Whittington L., Watson C., Johnson J., Musgrove J., Pence M., Hurley D., Baldwin C., Gardner I. A., Aly S.: Efficacy of spheroplastic and cell-wall competent vaccines for *Mycobacterium avium* subsp. paratuberculosis in experimentally-challenged baby goats. *Vet. Microbiol.* 2007, 120, 261-283.
8. Julian R. J.: A short review and some observations on Johne's disease with recommendations for control. *Can. Vet. J.* 1975, 16, 33-43.
9. Kawaji S., Begg D. J., Plain K. M., Whittington R. J.: A longitudinal study to evaluate the diagnostic potential of a direct faecal quantitative PCR test for Johne's disease in sheep. *Vet. Microbiol.* 2011, 148, 35-44.
10. Kirkeby C., Graesoll K., Nielsen S. S., Christiansen L. E., Toft N., Rattenborg E., Halasa T.: Simulating the epidemiological and economic impact of paratuberculosis control actions in dairy cattle. *Front. Vet. Sci.* 2016, 3, 90-102.
11. Krauze J., Monti G., Schulze F., Mella A., Leiva S.: Herd-level prevalence of Map infection in dairy herds of southern Chile determined by culture of environmental fecal samples and bulk-tank milk qPCR. *Prev. Vet. Med.* 2013, 111, 319-324.
12. Manning E. J., Collins M. T.: *Mycobacterium avium* subsp. paratuberculosis: pathogen, pathogenesis and diagnosis. *Rev. Sci. Tech.* 2001, 1, 133-150.
13. MacAloon C. G., Whyte P., O'Grady L., Lorenz I., Green M. G., Hogan I., Johnson A., Doherty M. L.: Relationship between selected perinatal paratuberculosis management interventions and passive transfer of immunity in dairy calves. *Vet. Record* 2016, 179, 47-53.
14. McDonald W. L., Ridge S. E., Hope A. F., Condon R. J.: Evaluation of diagnostic tests for Johne's disease in young cattle. *Aust. Vet. J.* 1999, 77, 113-119.

15. Merkal R. S.: Paratuberculosis: prevalence, diagnosis, prevention, and treatment. Proc. 17th Ann. Convention Am. Ass. Bovine Pract. 1985, 64, 66.
16. Millar D. S., Withey S. J., Tizard M. L., Ford J. G., Hermon-Taylor J.: Solid-phase hybridization capture of low-abundance target DNA sequences: application to the polymerase chain reaction detection of *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp. *silvaticum*. Anal. Biochem. 1995, 226, 325-330.
17. Mitchell R. M., Medley G. F., Collins M. T., Schukken Y. H.: A meta-analysis of the effect of dose and age at exposure on shedding of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in experimentally infected calves and cows. Epidemiol. Infect. 2012, 140, 231-246.
18. Mortier R. A. R., Barkema H. W., Orsel K., Wolf R., De Buck J.: Shedding patterns of dairy calves experimentally infected with *Mycobacterium avium* subspecies *paratuberculosis*. Vet. Res. 2014, 45, 71-79.
19. Nielsen S. S., Bjerre H., Toft N.: Colostrum and milk as risk factors for infection with *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle. J. Dairy Sci. 2008, 91, 4610-4615.
20. Pavlik I., Matlova L., Bartl J., Svastova P., Dvorska L., Whitlock R.: Parallel faecal and organ *Mycobacterium avium* subsp. *paratuberculosis* culture of different productivity types of cattle. Vet. Microbiol. 2000, 77, 309-324.
21. Pithua P., Espejo L. A., Godden S. M., Wells S. J.: Is an individual calving pen better than a group calving pen for preventing transmission of *Mycobacterium avium* subsp. *Paratuberculosis* in calves? Results from a field trial. Res. Vet. Sci. 2013, 95, 398-404.
22. Pithua P., Wells S. J., Godden S. M., Raizman E. A.: Clinical trial on type of calving pen and the risk of disease in Holstein calves during the first 90 d of life. Prev. Vet. Med. 2009, 89, 8-15.
23. Pithua P., Wells S. J., Sreevatsan S., Godden S. M.: Lack of evidence for fecal shedding of *Mycobacterium avium* subsp. *Paratuberculosis* in calves born to fecal culture positive dams. Prev. Vet. Med. 2010, 93, 242-245.
24. Riemann H. P., Abbas B.: Diagnosis and control of bovine paratuberculosis (Johne's disease). Adv. Vet. Sci. Comp. Med. 1983, 27, 481-506.
25. Roermund H. J. van, Bakker D., Willemsen P. T., de Jong M. C.: Horizontal transmission of *Mycobacterium avium* subsp. *Paratuberculosis* in cattle in an experimental setting: calves can transmit the infection to other calves. Vet. Microbiol. 2007, 122, 270-279.
26. Roermund H. J. W. van, de Jong M. C. M.: Horizontal transmission experiment of paratuberculosis. Proc. 8th Internat. Colloq. Paratuberculosis 2005, 672, 678.
27. Rossiter C. A., Burhans W. S.: Farm-specific approach to paratuberculosis (Johne's disease) control. Vet. Clin. N. Am. Food Anim. Pract. 1996, 12, 383-415.
28. Sherman D. M.: Unexplained weight loss in sheep and goats. A guide to differential diagnosis, therapy, and management. Vet. Clin. North Am. Large Anim. Pract. 1983, 5, 571-590.
29. Smith R. L., Schukken Y. H., Pradhan A. K., Smith J. M., Whitlock R. H., Kessel J. S. van, Wolfgang D. R., Grohn Y. T.: Environmental contamination with *Mycobacterium avium* subsp. *Paratuberculosis* in endemically infected dairy herds. Prev. Vet. Med. 2011, 102, 1-9.
30. Stabel J. R., Palmer M. V., Harris B., Plattner B., Hostetter J., Robbe-Austerman S.: Pathogenesis of *Mycobacterium avium* subsp. *paratuberculosis* in neonatal calves after oral or intraperitoneal experimental infection. Vet. Microbiol. 2009, 136, 306-313.
31. Subharat S., Shu D., Wedlock D. N., Price-Carter M., de Lisle G. W., Luo D., Collins D. M., Buddle B. M.: Immune responses associated with progression and control of infection in calves experimentally challenged with *Mycobacterium avium* subsp. *paratuberculosis*. Vet. Immunol. Immunopathol. 2012, 149, 225-236.
32. Sweeney R. W.: Pathogenesis of paratuberculosis. Vet. Clin. N. Am.: Food Anim. Pract. 2011, 27, 537-546.
33. Sweeney R. W., Whitlock R. H., Hamir A. N., Rosenberger A. E., Herr S. A.: Isolation of *Mycobacterium paratuberculosis* after oral inoculation in uninfected cattle. Am. J. Vet. Res. 1992, 53, 1312-1314.
34. Tiwari A., Vanleeuwen J. A., Dohoo J. A., Keefe G. P., Haddad J. P., Scott H. M., Whiting T.: Risk factors associated with *Mycobacterium avium* subsp. *Paratuberculosis* seropositivity in Canadian dairy cows and herds. Prev. Vet. Med. 2009, 88, 32-41.
35. Weber M. F., Kogut J., de Bree J., van Schaik G.: Evidence of *Mycobacterium avium* subsp. *paratuberculosis* shedding in young cattle. Proc. 8th Internat. Colloq. Paratuberculosis 2005, 679, 689.
36. Weber M. F., Kogut J., de Bree J., van Schaik G., Nielen M.: Age at which dairy cattle become *Mycobacterium avium* subsp. *paratuberculosis* faecal culture positive. Prev. Vet. Med. 2010, 97, 29-36.
37. Wells S. J.: The prospects for herd level control of paratuberculosis – a scientists view. Proc. 8th Internat. Colloq. Paratuberculosis 2005, 174, 181.
38. Wells S. J., Wagner B. A.: Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J. Am. Vet. Med. Assoc. 2000, 216 1450-1457.
39. Whitlock R. H., Hutchinson L. T., Glickman L. T., Meinersmann R., Rossiter C., Bruce J., Merkal R., Dick J.: Paratuberculosis (Johne's disease) update. Bovine Pract. 1986, 21, 24-30.
40. Whitlock R. H., Wells S. J., Sweeney R. W., Van Tiem J.: ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet. Microbiol. 2000, 77, 387-398.
41. Windsor P. A., Whittington R. J.: Evidence for age susceptibility of cattle to Johne's disease. Vet. J. 2010, 1, 37-44.
42. Wiszniewska-Laszczych A., Sztejn J., Ruszczyńska A.: A case study of the clinical form of Johne's disease in a heifer. Turk. J. Vet. Anim. Sci. 2010, 1, 95-99.
43. Woodbine K. A., Schukken Y. H., Green L. E., Ramirez-Villaescusa A., Mason S., Moore S. J., Bilbao C., Swann N., Medley G. F.: Seroprevalence and epidemiological characteristics of *Mycobacterium avium* subsp. *paratuberculosis* on 114 cattle farms in south west England. Prev. Vet. Med. 2009, 89, 102-109.

Adres autora: Dr Agnieszka Wiszniewska-Laszczych, PhD; e-mail: aga@uwm.edu.pl