Acta Veterinaria Hungarica 64 (3), pp. 301–312 (2016)
DOI: 10.1556/004.2016.029

EFFECTS OF SUBCLINICAL *MYCOBACTERIUM AVIUM* SSP. *PARATUBERCULOSIS* INFECTION ON SOME PHYSIOLOGICAL PARAMETERS, HEALTH STATUS AND PRODUCTION IN DAIRY COWS

Viktor JURKOVICH[1*], Barbara BOGNÁR[1], Krisztián BALOGH[2], Mária KOVÁCS-WEBER[3], Kinga FORNYOS[4], Rubina Tünde SZABÓ[3], Péter KOVÁCS[1], László KÖNYVES[1] and Miklós MÉZES[2]

[1]Department of Animal Hygiene, Herd Health and Veterinary Ethology, University of Veterinary Medicine, István utca 2, H-1078 Budapest, Hungary; [2]Department of Nutrition, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, Hungary; [3]Institute of Animal Husbandry, Faculty of Agricultural and Environmental Science, Szent István University, Gödöllő, Hungary; [4]M.A.H Food-Control Ltd., Vet-Control Laboratory, Budapest, Hungary

(Received 28 April 2016; accepted 15 July 2016)

Milk yield, milk ingredients, health and other, production-related parameters of subclinically infected, *Mycobacterium avium* ssp. *paratuberculosis* (MAP-) shedding (positive faecal PCR, n = 20) and non-shedding (negative faecal PCR, n = 10) dairy cows were compared in the period from 10 days prepartum to 120 days postpartum. Body condition, rumen fill and faeces scores were lower in the MAP-shedding cows. There was no significant difference in plasma or urine metabolic parameters between the groups. Milk yield and lactose content tended to be lower (P = 0.074 and 0.077, respectively), somatic cell count tended to be higher (P = 0.097), while milk fat content was significantly higher (P = 0.006) in MAP-shedding cows than in the controls. Milk protein content did not differ between the groups. All other health and production parameters [number of reproductive tract treatments, number of udder treatments, number of artificial inseminations (AIs), calving interval, and service period] were significantly better in the control group. It is concluded that MAP infection, even in a subclinical form, has a significant impact on some production and health parameters of dairy cows.

Key words: Paratuberculosis, metabolic parameters, milk yield, reproduction, dairy cow

Paratuberculosis (Johne's disease, JD) is the chronic inflammation of the colonic mucosa in ruminants, caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). The pathogen can remain viable and infective for up to 12 months in a dry, fully shaded environment (Donat et al., 2016). Wild animals can also be

*Corresponding author; E-mail: jurkovich.viktor@univet.hu; Phone: 0036 (1) 478-4242; Fax: 0036 (1) 478-4243

infected and serve as reservoirs (Garcia et al., 2008). According to a recent review (Garcia and Shalloo, 2015), the apparent prevalence in dairy herds is reported be 0.52–41.4% on animal level and 7–83.3% on herd level. True prevalence estimates are rarely obtained.

The disease usually develops to the clinical stage in the second or third lactation, with extremes of 4 months to 15 years of age (Garcia and Shalloo, 2015). The agent can be transmitted with faeces and milk, even in the subclinical phase of the disease (Beaudeau et al., 2007). Airborne infection via dust particles was also described (Eisenberg et al., 2011). Barn hygiene, especially in the calving pen, is crucial in the prevention of new infections (Sweeney et al., 2012; Donat et al., 2016).

Research suggests that MAP might present a zoonotic risk due to its potential association with Crohn's disease in humans (Chiodini et al., 2012). Implementing control programs to reduce spread within and between herds are therefore of fundamental importance (Geraghty et al., 2014). The most common methods in JD monitoring are faecal PCR assays, which proved to be more sensitive than ELISA (Nielsen et al., 2002).

The disease causes serious economic loss to the dairy industry, such as decreased milk yield, hindered feed conversion, lower fertility, and other disorders (Garcia and Shalloo, 2015). Reduced slaughter weight and early culling are also reported (Fodor et al., 2014; Pieper et al., 2015). Several studies have estimated the farm-level economic loss of MAP infections to be in the range of EUR 35–165 per cow (Stott et al., 2005; Tiwari et al., 2008; Fodor et al., 2014).

The aim of present study was to examine the effects of subclinical MAP infections on the health as well as on some clinical biochemical parameters and production traits of high-yielding dairy cows in a Hungarian dairy herd.

Materials and methods

Screening of dairy herds

Bulk-tank milk samples were collected for PCR assay in 2014 and 2015 from 29 large-scale dairy farms in Hungary to screen the MAP status of the herds. The only farm with a positive sample was chosen for further studies.

Pooled faecal samplings were performed with five animals/pool, and faecal samples of the animals in the positive pools were then analysed individually.

For DNA extraction, the NucleoSpin® Tissue Kit (Macherey-Nagel, Germany) was used. The fractions were analysed through real-time PCR (RT-PCR) using the MX3000P of Stratagene RT-PCR system (Thermo Fisher Scientific, USA), and for the detection of MAP Adiavet™ ParaTB Real Time PCR test (bioMérieux, France) was used.

Experimental animals, sampling protocol and data collection

Health and production parameters were monitored around the time of calving and through the first semester of lactation and cows in the dry period were also involved in the experiment. A total of 15 cows that were MAP positive by PCR were allocated in the experimental group but none of them showed clinical signs of paratuberculosis, and 15 cows that were negative for both faecal PCR and clinical signs were selected as controls. At the end of the experimental period the MAP status of the cows was re-evaluated. Five animals from the control group were found to be MAP positive, and reallocated in the experimental group. The statistical analysis therefore involved 20 animals as MAP shedders (MAP+, age: 5.6 ± 1.6 years; lactation: 3.5 ± 1.4; BCS: 3.0 ± 0) and 10 animals as control (CO, age: 5.2 ± 2.8 years; lactation: 3.4 ± 2.2, BCS: 3.5 ± 0.3).

Experimental and control cows were not housed or treated separately from other animals during the period of investigation. The housing and feeding conditions were identical for MAP+ and control cows. During lactation the animals were fed total mixed ration (TMR) twice a day and milked four times daily. Drinking water was available *ad libitum.*

Blood and urine samples were taken between January and July 2015, from day 10 prior to the expected date of calving until day 120 of lactation (Table 1). Blood and urine samples were taken 3–5 h after the morning feeding.

Table 1

Sampling schedule followed during the experiment

Sampling	MAP+ and CO
1	D 10–14 prepartum
2	D 2–5 pp.
3	D 10 pp.
4	D 20 pp.
5	D 30 pp.
6	D 40 pp.
7	D 50 pp.
8	D 60 pp.
9	D 80 pp.
10	D 100 pp.
11	D 120 pp.

CO = control; pp. = postpartum

Plasma samples were analysed for total protein (TP), albumin, total cholesterol (CH), triglyceride (TG), beta-hydroxybutyrate (BHB), nonesterified fatty acids (NEFA), urea, total calcium, inorganic phosphate, and carotene concentrations. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) were also measured. These

analyses were performed with a BMG Labtech SPECTROstar Nano (BMG Labtech, Germany) biochemistry analyser using commercial kits (Diagon Ltd., Hungary for BHB, Randox Ltd., Ireland for NEFA, and Diagnosticum Ltd., Hungary for all others).

The pH of the urine samples was measured with a digital pH meter (Radelkis OP-211/1, Radelkis Co., Hungary), and net acid-base excretion (NABE) was determined according to the method of Kutas (1965).

At the time of the blood samplings the body condition of the animals was scored (BCS) on a 1 to 5 scale (Mulvany, 1977). Rumen fill and faeces consistency (Zaaijer and Noordhuizen, 2003) were also scored at the same time.

Monthly test-day milk recording data and certain health and reproduction-related parameters were collected throughout the lactation and obtained from the farm database (Riska™ by Systo, Hungary).

Statistical analysis

Statistical analysis was performed using the R 3.2.3 statistical software (R Core Team, 2015). For the analysis of production traits, the Shapiro-Wilk test was used to test the normality of data, and in case of normal distribution a paired *t*-test, while in case of non-normality the Wilcoxon rank sum test was used to test the difference between groups. For the metabolic parameters, BCS, rumen fill and faeces scores a general linear model was used to calculate the effects between groups, individuals or samplings. Pearson correlations between some parameters were also established. The level of significance was set to $P < 0.05$.

Results

Metabolic parameters

The results of the blood and urine parameters, body condition, rumen fill and faeces scoring are shown in Table 2.

The body condition, rumen fill and faeces scores differed significantly between the two groups, all of them being lower in the MAP+ cows. There was no significant difference in the plasma or urine metabolic parameters between the groups. There were moderate but significant correlations between BCS and rumen fill ($r = 0.23$, $P = 0.001$) as well as between BCS and faeces score ($r = 0.41$, $P < 0.001$). The correlations between BCS and BHB ($r = –0.14$, $P = 0.059$) or NEFA ($r = 0.07$, $P = 0.307$) were low and not significant.

Production and health-related parameters

The results obtained on production and health-related parameters are summarised in Table 3.

Table 2

Mean (± SD) values of metabolic parameters, body condition score (BCS), rumen fill and faeces scores in MAP-shedding (MAP+) and control (CO) groups

Item	MAP+ n = 20	CO n = 10	P value
BCS	2.3 ± 0.5	2.8 ± 0.3	< 0.001
Rumen fill score	2.4 ± 0.6	2.7 ± 0.5	< 0.001
Faeces score	2.0 ± 0.6	2.5 ± 0.5	< 0.001
Blood plasma			
BHB (mmol/L)	0.7 ± 0.4	0.6 ± 0.3	0.405
NEFA (mmol/L)	0.3 ± 0.2	0.3 ± 0.2	0.775
AST (U/L)	39 ± 15	37 ± 17	0.445
GGT (U/L)	19 ± 8	18 ± 5	0.057
ALT (U/L)	17 ± 6	18 ± 6	0.392
Total protein (g/L)	101 ± 14	94 ± 14	0.215
Albumin (g/L)	34 ± 4	34 ± 4	0.939
Cholesterol (mmol/L)	5.3 ± 1.7	5.7 ± 2.2	0.057
Triglycerides (mmol/L)	0.11 ± 0.07	0.12 ± 0.09	0.737
Ca (mmol/L)	2.1 ± 0.4	2.1 ± 0.4	0.580
Inorganic P (mmol/L)	1.8 ± 0.4	1.9 ± 0.3	0.075
Urea (mmol/L)	7.8 ± 2.2	8.0 ± 1.6	0.407
Carotene (µmol/L)	2.7 ± 0.9	2.8 ± 1.4	0.483
Urine			
pH	8.4 ± 0.2	8.4 ± 0.1	0.515
NABE (mmol/L)	186 ± 62	199 ± 56	0.135

Table 3

Mean (± SD) of production and health-related parameters in MAP-shedding (MAP+) and control (CO) groups

Item	MAP+ n = 20	CO n = 10	P value
Production parameters			
Milk yield (L)	47.5 ± 12.3	51.9 ± 9.3	0.074
Milk fat (%)	3.6 ± 0.7	3.2 ± 0.7	0.006
Milk protein (%)	3.1 ± 0.3	3.1 ± 0.3	0.509
Lactose (%)	4.9 ± 0.2	5.0 ± 0.2	0.077
Somatic cell count (10^3/ml)	1036 ± 1077	697 ± 1162	0.097
Health- and reproduction-related parameters			
Number of reproductive tract treatments	5.7 ± 4.3	2.4 ± 1.9	< 0.001
Number of udder treatments	2.9 ± 2.5	0.6 ± 0.7	0.003
Number of AIs	2.8 ± 1.6	1.4 ± 0.5	0.015
Calving interval (days)	437 ± 66	365 ± 33	0.006
Service period (days)	168.9 ± 72.3	84.6 ± 33.1	0.003
Pregnancy rate (%) to the 1st AI	22.5 ± 27.2	37.2 ± 31.8	0.114

Milk yield and lactose content tended to be lower (P = 0.074 and 0.077, respectively), somatic cell count (SCC) tended to be higher (P = 0.097), while milk fat content was significantly higher (P = 0.006) in MAP+ cows than in the controls. However, milk protein content did not differ between the groups.

All other health and production parameters, except for pregnancy rate to the first artificial insemination (AI), were significantly better in the CO group.

Discussion

Rónai et al. (2015) reported a 2.4–14.1% animal-level prevalence of MAP infections in Hungary. MAP is present throughout the country in cow populations, and also in wild animals (wild boar, red deer, red fox, water buffalo). The bulk tank milk positivity, as detected by PCR, varies among studies (Sweeney et al., 2012). Khol et al. (2013) found no MAP-positive bulk tank milk samples in their study, while others reported a rate similar to our results (3.4%; Beaver et al., 2016), or even as high as 52% positive samples (Stabel et al., 2002).

Subclinical infections usually remain undiagnosed. Animals without clinical signs are not culled and continue to spread the pathogen, thus increasing the infection rate within the herd (Clarke, 1997). Additionally, many MAP-infected animals can show a latent or intermittent shedding stage where no MAP isolated can be found using the current diagnostic methods (Schukken et al., 2015), as it was seen in our study, namely that some MAP-negative animals were rearranged to the MAP+ group at the end of the experimental period.

In the present study there was no difference in metabolic parameters between MAP+ and control cows. This finding was rather surprising, since an impaired metabolic status was presumed in the MAP+ group, especially as there were differences between the two groups in BCS, rumen fill and faeces scores (Table 2). At least some level of hypoproteinaemia or signs of energy imbalance were hypothesised. We suppose that this finding is due to the early stage of MAP infection without any morphologic changes in the gut mucosa. Limited information is available about the metabolic status and changes in blood or urine biochemical parameters in cows shedding MAP. Contrary to our results, Donat et al. (2014*a*) reported a decreased total protein (TP) level in the serum samples of MAP+ cows, including those shedding a low number of bacteria and presumably being in a subclinical phase of paratuberculosis. In conformity with our results, they did not find differences in BHB, bilirubin and cholesterol levels and ALT activity in MAP-shedding cows. Earlier studies also reported lower levels of TP in subclinically infected calves (Szilágyi et al., 1989; Körmendy et al., 1990). McGregor et al. (2015) reported that several biochemical parameters, except serum albumin, were similar between MAP-positive and MAP-negative Merino sheep. The serum albumin concentration was lower in sheep having more serious

histopathology scores and lower body weight. The clinical cases were not excluded in their study, and serum albumin level was similar in sheep with mild (1 and 2) intestinal lesions (maybe subclinical cases) as in the controls. Fodor et al. (2014) reported a higher rate of early culling among MAP+ cows, mostly due to metabolic problems; however, this finding was not verified by the results of blood samplings and biochemical analysis.

The acceptable BCS of cows at the time of calving is 3.0–3.5 (on a 5-point scoring scale), which is expected to decrease by one point in the early milk production period (Roche et al., 2009). Body condition scores started from the ideal 3.0–3.5 at calving in our study, but the rate of decrease of body condition was different in the two groups (Fig. 1), possibly indicating the effect of MAP infection. It has also been reported that JD causes a significant body weight loss in cows (Fodor et al., 2014) and sheep (McGregor et al., 2015); yet, to the best of our knowledge, there are no data about the effect of subclinical MAP infection on the BCS in dairy cows.

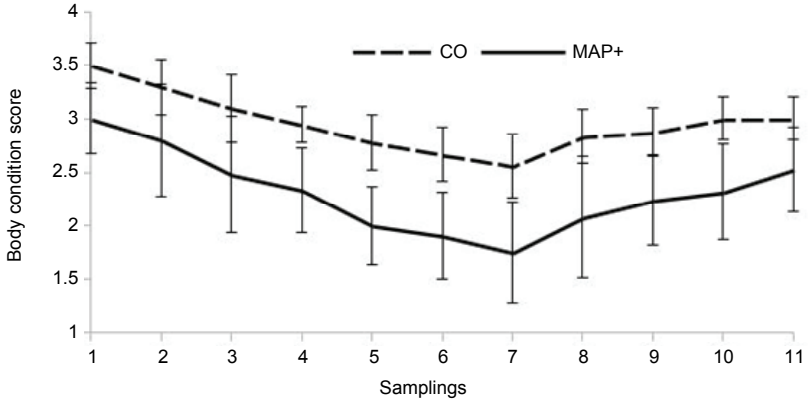

Fig. 1. Body condition score of the cows examined

The faeces score was lower in MAP-shedding animals, although diarrhoea was not observed in any of the animals examined. We found that the lowest faeces scores were obtained at the time of the third sampling (D 10 postpartum, data not shown), but this was possibly caused by the relatively high ratio of concentrate in the TMR at the onset of lactation. Based on these findings it can be suggested that feed conversion – and primarily that of the readily fermentable carbohydrates – is impaired in MAP+ animals, resulting in more liquid faecal consistency. However, further studies are needed to confirm this assumption.

All of the examined reproduction parameters differed between the groups, which suggests that even a subclinical MAP infection can significantly hinder the reproductive success of the herd. In the CO group, the reproduction parameters were better than the herd average, while the MAP+ group revealed worse

values. Similarly, in the study of Johnson-Ifearulundu et al. (2000) the calving interval was on average 27.9 days longer in MAP+ cows than in the control animals.

The number of reproductive tract treatments was higher in the MAP+ cows in our study, resulting in higher veterinary and treatment costs in the case of JD (Benedictus et al., 1987; Fodor et al., 2014), although we did not quantify the economic effects of subclinical MAP infection.

According to the study of Weiss et al. (2006), there is a general hyporesponsiveness of the cellular immune system in MAP-infected cows. Antibody production is also severely decreased in the infected animals (Kreeger et al., 1991). Due to the impaired cellular immune response, subclinical paratuberculosis makes animals more susceptible to infections. MAP infection and immunodeficiency may cause secondary diseases, including reproductive and udder problems, that often lead to the culling of animals (Johnson-Ifearulundu and Kaneene, 1997).

The milk yield tended to be lower in the MAP+ group. There are several studies analysing the effects of MAP infection on the milk yield of dairy cows where a decrease in milk yield is reported (Benedictus et al., 1987; Wilson et al., 1993; Nordlund et al., 1996). The decrease in the milk production of subclinically infected cows tends to be progressive, but statistical differences may only be confirmed in the fourth or later lactations (Tiwari et al., 2007, 2008). However, other authors did not find any difference (Johnson et al., 2001) or even reported an increased milk yield (McNab et al., 1991) in MAP-infected cows. According to a recent meta-analysis by McAloon et al. (2016), the calculated combined effect of MAP infection was –1.87 kg milk/cow per day, estimated to correspond to a 5.9% decrease in milk yield, which is lower than the results of the present study. According to Donat et al. (2014*b*), the decrease in milk production of MAP-positive cattle depends on the within-herd prevalence.

The higher milk fat percent in the MAP+ group cannot be explained on the basis of the known pathogenesis of the disease and the hypothesised digestive and metabolic disorders. The effect of infection on milk composition is not fully understood. Gonda et al. (2007) found that MAP+ cows produce less milk fat and protein, while Pillars et al. (2011) and Donat et al. (2014*b*) could not demonstrate such an effect.

The udder health of MAP+ cows is reflected in the number of udder treatments and the higher tendency of somatic cell count in this group. The number of treatments was 4.8-fold higher in the MAP+ cows in the first 120 days of lactation. Several studies have demonstrated that chronic mastitis is one of the main causes of early culling of MAP+ dairy cows (Dufour et al., 2004; McSpadden et al., 2013; Garcia and Shalloo, 2015); however, other authors found no correlation between MAP status and SCC (Gonda et al., 2007; Donat et al., 2014*b*).

These contradicting results possibly derive from the inaccuracy or alternative application of diagnostic methods (Pillars et al., 2011). If higher numbers of animals are shedding the pathogen, more pronounced effects can be found in the production traits (Lombard et al., 2005; Raizman et al., 2009). Production loss is more severe in animals positive by faecal PCR than in those positive by ELISA (Gonda et al., 2007). According to Smith et al. (2016), low-pathology animals (having only at least one positive culture or positive ELISA result) were shown to recover some productivity, while high-pathology animals (at least one high-positive culture) continued to exhibit a production decrease.

It can be concluded that MAP infection, even in a subclinical form, has a significant impact on the production, some reproductive parameters and the udder health of cows. A more exact estimation of its economic impact might provide an insight into the cost efficiency of screening for subclinical infections, which would help prevent new infections and improve herd health and productivity.

Acknowledgements

This study was supported by a grant from the National Research, Development and Innovation Office (Grant No.: KMR_12-1-2012-0161). The research was supported by the 11475-4/2016/FEKUT and 11476-3/2016/FEKUT grants of the Hungarian Ministry of Human Resources.

References

Beaudeau, F., Belliard, M., Joly, A. and Seegers, H. (2007): Reduction in milk yield associated with *Mycobacterium avium* subspecies *paratuberculosis* (Map) infection in dairy cows. Vet. Res. **38,** 625–634.

Beaver, A., Cazer, C. L., Ruegg, P. L., Gröhn, Y. T. and Schukken, Y. H. (2016): Implications of PCR and ELISA results on the routes of bulk-tank contamination with *Mycobacterium avium* ssp. *paratuberculosis*. J. Dairy Sci. **99,** 1391–1405.

Benedictus, G., Dijkhuzien, A. A. and Stelwagen, J. (1987): Economic losses due to paratuberculosis in dairy cattle. Vet. Rec. **121,** 142–146.

Chiodini, R. J., Chamberlin, W. M., Sarosiek, J. and McCallum, R. W. (2012): Crohn's disease and the mycobacterioses: a quarter century later. Causation or simple association? Crit. Rev. Microbiol. **38,** 52–93.

Clarke, C. J. (1997): The pathology and pathogenesis of paratuberculosis in ruminants and other species. J. Comp. Pathol. **116,** 217–261.

Donat, K., Erhardt, G., Soschinka, A. and Brandt, H. R. (2014*a*): Decreased serum protein associated with *Mycobacterium avium* subspecies *paratuberculosis* shedding in German Holstein cows. Vet. Rec. **174,** 408.

Donat, K., Soschinka, A., Erhardt, G. and Brandt, H. R. (2014*b*): Paratuberculosis: decrease in milk production of German Holstein dairy cows shedding *Mycobacterium avium* ssp. *paratuberculosis* depends on within-herd prevalence. Animal **8,** 852–858.

Donat, K., Schmidt, M., Köhler, H. and Sauter-Louis, C. (2016): Management of the calving pen is a crucial factor for paratuberculosis control in large dairy herds. J. Dairy Sci. **99,** 3744–3752.

Dufour, B., Pouillot, R. and Durand, B. (2004): A cost/benefit study of paratuberculosis certification in French cattle herds. Vet. Res. **35,** 69–81.

Eisenberg, S. W., Koets, A. P., Nielen, M., Heederik, D., Mortier, R., De Buck, J. and Orsel, K. (2011): Intestinal infection following aerosol challenge of calves with *Mycobacterium avium* subspecies *paratuberculosis*. Vet. Res. **42,** 117.

Fodor, I., Matyovszky, B., Biczó, A. and Ózsvári, L. (2014): The losses due to paratuberculosis and its control in a Hungarian large-scale Holstein-Friesian dairy farm [in Hungarian, with English abstract]. Magy. Allatorvosok **136,** 213–222.

Garcia, A. B. and Shalloo, L. (2015): The economic impact and control of paratuberculosis in cattle. J. Dairy Sci. **98,** 5019–5039.

Garcia, R., Perez-de-la-Lastra, J. M., Vicente, J., Ruiz-Fons, F., Garrido, J. M. and Gortazar, C. (2008): Large-scale ELISA testing of Spanish red deer for paratuberculosis. Vet. Immunol. Immunopathol. **124,** 75–81.

Geraghty, T., Graham, D. A., Mullowney, P. and More, S. J. (2014): A review of bovine Johne's disease control activities in 6 endemically infected countries. Prev. Vet. Med. **116,** 1–11.

Gonda, M. G., Chang, Y. M., Shook, G. E., Collins, M. T. and Kirkpatrick, B. W. (2007): Effect of *Mycobacterium paratuberculosis* infection on production, reproduction, and health traits in US Holsteins. Prev. Vet. Med. **80,** 103–119.

Johnson, Y. J., Kaneene, J. B., Gardiner, J. C., Lloyd, J. W., Sprecher, D. J. and Coe, P. H. (2001): The effect of subclinical *Mycobacterium paratuberculosis* infection on milk production in Michigan dairy cows. J. Dairy Sci. **84,** 2188–2194.

Johnson-Ifearulundu, Y. J., Kaneene, J. B., Sprecher, D. J., Gardiner, J. C. and Lloyd, J. W. (2000): The effect of subclinical *Mycobacterium paratuberculosis* infection on days open in Michigan, USA, dairy cows. Prev. Vet. Med. **46,** 171–181.

Johnson-Ifearulundu, Y. J. and Kaneene, J. B. (1997): Epidemiology and economic impact of subclinical Johne's disease: A review. Vet. Bull. **67,** 437–447.

Khol, J. L., Wassertheurer, M., Sodoma, E., Revillia-Fernandez, E., Damoser, J., Österreicher, E., Dünster, M., Kleb, U. and Baumgartner, W. (2013): Long-term detection of *Mycobacterium avium* subspecies *paratuberculosis* in individual and bulk tank milk from a dairy herd with a low prevalence of Johne's disease. J. Dairy Sci. **96,** 3517–3524.

Körmendy, B., Szilágyi, M., Tuboly, S. and Nagy, Gy. (1990): Some diagnostic features of the pathogenesis of bovine paratuberculosis (Johne's disease) and serum biochemical changes after oral reinfection. J. Vet. Med. B **37,** 229–235.

Kreeger, J. M., Snider, T. G. and Olcott, B. M. (1991): Spontaneous murine thymocyte comitogenic activity consistent with interleukin-1 in cattle naturally infected with *Mycobacterium paratuberculosis.* Vet. Immunol. Immunopathol. **28,** 317–326.

Kutas, F. (1965): The measurement of net acid base excretion in the urine of cattle (A method for the estimation of acid–base equilibrium) [in Hungarian, with English abstract]. Magy. Allatorvosok **20,** 104–107.

Lombard, J. E., Garry, F. B., McCluskey, B. J. and Wagner, B. A. (2005): Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. J. Am. Vet. Med. Assoc. **227,** 1975–1981.

McAloon, C. G., Whyte, P., More, S. J., Green, M. J., O'Grady, L., Garcia, A. B. and Doherty, M. L. (2016): The effect of paratuberculosis on milk yield – A systematic review and meta-analysis. J. Dairy Sci. **99,** 1449–1460.

McGregor, H., Abbott, K. A. and Whittington, J. R. (2015): Effects of *Mycobacterium avium* subsp. *paratuberculosis* infection on serum biochemistry, body weight and wool growth in Merino sheep: A longitudinal study. Small Rumin. Res. **125,** 146–153.

McNab, W. B., Meek, A. H., Martin, S. W. and Duncan, J. R. (1991): Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. Can. J. Vet. Res. **55,** 356–361.

McSpadden, K., Caires, K. and Zanella, R. (2013): The effect of *Mycobacterium avium* subspecies *paratuberculosis* exposure on animal health. Acta Sci. Vet. **41,** 1095.

Mulvany, P. (1977): Dairy cow condition scoring. NIRD Paper No. 4468, National Institute for Research in Dairying, Reading, UK.

Nielsen, S. S., Gronbak, C., Agger, J. F. and Houe, H. (2002): Maximum-likelihood estimation of sensitivity and specificity of ELISAs and faecal culture for diagnosis of paratuberculosis. Prev. Vet. Med. **53,** 191–204.

Nordlund, K. V., Goodger, W. J., Pelletier, J. and Collins, M. T. (1996): Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. J. Am. Vet. Med. Assoc. **208,** 1872–1876.

Pieper, L., Sorge, U. S., DeVries, T. J., Godkin, A., Lissemore, K. and Kelton, D. F. (2015): Evaluation of the Johne's disease risk assessment and management plan on dairy farms in Ontario, Canada. J. Dairy Sci. **98,** 6792–6800.

Pillars, R. B., Bolton, M. W. and Grooms, D. L. (2011): Case-control study: productivity and longevity of dairy cows that tested positive for infection with *Mycobacterium avium* ssp. *paratuberculosis* as heifers compared to age-matched controls. J. Dairy Sci. **94,** 2825–2831.

R Core Team (2015): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Raizman, E. A., Fetrow, J. P. and Wells, S. J. (2009): Loss of income from cows shedding *Mycobacterium avium* subspecies *paratuberculosis* prior to calving compared with cows not shedding the organism on two Minnesota dairy farms. J. Dairy Sci. **92,** 4929–4936.

Roche, J. R., Friggens, N. C., Kay, J. K., Fisher, M. W., Stafford, K. J. and Berry, D. P. (2009): Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. **92,** 5769–5801.

Rónai, Zs., Csivincsik, Á., Szőgyényi, Zs., Bacsadi, Á., Dán, Á. and Jánosi, Sz. (2015): Data on the occurrence of paratuberculosis in Hungary – diagnostic improvements and results from 2006–2012 [in Hungarian, with English abstract]. Magy. Allatorvosok **137,** 211–218.

Schukken, Y. H., Whitlock, R. H., Wolfgang, D., Grohn, Y., Beaver, A., VanKessel, J., Zurakowski, M. and Mitchell, R. (2015): Longitudinal data collection of *Mycobacterium avium* subspecies *paratuberculosis* infections in dairy herds: the value of precise field data. Vet. Res. **46,** 65.

Smith, R. L., Gröhn, Y. T., Pradhan, A. K., Whitlock, R. H., Van Kessel, J. S., Smith, J. M., Wolfgang, D. R. and Schukken, Y. H. (2016): The effects of progressing and nonprogressing *Mycobacterium avium* ssp. *paratuberculosis* infection on milk production in dairy cows. J. Dairy Sci. **99,** 1383–1390.

Stabel, J. R., Wells, S. J. and Wagner, B. J. (2002): Relationships between fecal culture, ELISA, and bulk tank milk test results for Johne's disease in US dairy herds. J. Dairy Sci. **85,** 525–531.

Stott, A. W., Jones, G. M., Humphry, R. W. and Gunn, G. J. (2005): Financial incentive to control paratuberculosis (Johne's disease) on dairy farms in the United Kingdom. Vet. Rec. **156,** 825–831.

Szilágyi, M., Körmendy, B., Suri, A., Tuboly, S. and Nagy, Gy. (1989): Experimental paratuberculosis (Johne's disease) – studies on biochemical parameters in cattle. Arch. Exper. Vet. Med. **43,** 463–470.

Sweeney, R. W., Collins, M. T., Koets, A. P., McGuirk, S. M. and Roussel, A. J. (2012): Paratuberculosis (Johne's disease) in cattle and other susceptible species. J. Vet. Intern. Med. **26,** 1239–1250.

Tiwari, A., VanLeeuwen, J. A. and Haddad, J. P. (2007): Production effects of seropositivity of pathogens causing bovine leucosis, bovine viral diarrhea, paratuberculosis, and neosporosis. J. Dairy Sci. **90,** 659–669.

Tiwari, A., VanLeeuwen, J. A., Dohoo, I. R., Keefe, G. P. and Weersink, A. (2008): Estimate of the direct production losses in Canadian dairy herds with subclinical *Mycobacterium avium* subspecies *paratuberculosis* infection. Can. Vet. J. **49,** 569–576.

Weiss, D. J., Evanson, O. A. and Souza, C. D. (2006): Mucosal immune response in cattle with subclinical Johne's disease. Vet. Pathol. **43,** 127–135.

Wilson, D. J., Rossiter, C., Han, H. R. and Sears, P. M. (1993): Association of *Mycobacterium paratuberculosis* infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. Am. J. Vet. Res. **54,** 1851–1857.

Zaaijer, D. and Noordhuizen, J. T. M. (2003): A novel scoring system for monitoring the relationship between nutritional efficiency and fertility in dairy cows. Irish J. Vet. Sci. **56,** 145–151.