Infertility in dairy cows – Possible bacterial and viral causes

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Abstract

In this research uterine swab and biopsy samples were collected from 40 infertile dairy cows kept at five dairy cattle farms in Hungary. Samples were tested for bacteria including *Coxiella burnetii* chlamydiae*, Mycoplasma* and *Ureaplasma*, and for the viruses Bovine herpesvirus 1 (BoHV-1) and Bovine viral diarrhoea virus (BVDV). *Chlamydiaceae* DNA was detected by real-time PCR in 22/40 (55%) samples. *Coxiella burnetii* DNA was detected in 3/40 (7.5%) cases by real-time PCR. *Mycoplasma* and *Ureaplasma* DNA was found in 2/40 (5%) and 4/40 (10%) cows, respectively. BVD and BoHV-1 DNA was not detected in any samples. *Escherichia coli* as a recognised uterine pathogen was found in two cases. The following potential uterine pathogens were found: *Bacillus licheniformis* (one case), non-haemolytic streptococci (five cases), *Histophilus somni* (two cases) and *Candida krusei* (two cases). Blood samples were collected at same time as swab samples from all 40 cows, and their examination for *C. burnetii* antibodies by ELISA revealed seropositivity in 26/40 cows (65%). Histological examination of the uterine biopsy samples showed the presence of mild lympho-histiocytic infiltration in the mucosa in 22 cases (59%). Moderate lympho-histiocytic infiltration of the endometrium was evident in 13 cases (35%), while in two cases (6%) severe inflammatory cell infiltration of the endometrium with lympho-histiocytes and neutrophil granulocytes was found. Although no statistical correlation could be demonstrated between the severity of histological lesions of the endometrium and the uterine pathogenicity of the bacteria (*P* = 0.8555), endometritis of a certain severity grade and/or a recognised or potential uterine pathogen were found in all samples. The latter may play a role in the development of infertility either collectively or independently.

Key words: *Bovine infertility; Dairy cow; Histological examination; Uterine biopsy; Uterine swab*

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Introduction

Reproductive disorders are the second largest source of economic loss (after mastitis (Benić et al., 2018; Burović, 2020)) and the number one cause of involuntary culling among herd-level animal health problems in dairy herds (Ózsvári and Kerényi, 2004; Bonneville-Hébert et al., 2011; Folnožić et al., 2015; Kovács et al., 2020). Up to 40% of culled cows are removed from the herd because of infertility (Meadows et al., 2005). The most important economic consequences of suboptimal reproductive performance are the increased calving interval (leading to decreased milk production per year), higher culling risk, decreased calf sales, and increased treatment and semen costs (Cabrera, 2014; Folnožić et al., 2019). The role of several bacterial, viral and protozoal agents of infertility of dairy cattle has been reviewed (Yoo, 2010; Wathes et al., 2020), confirming their importance as sources of economic loss.

As regards viral infections, the average financial loss caused by a cow seropositive for bovine herpesvirus-1 (BoHV-1) was estimated at USD 379, compared to their seronegative counterparts (Can et al., 2016). This loss increased to USD 509 per case in the case of BoHV-1-induced abortion. De Vries (2006) estimated the average cost of any abortion at USD 555. The analysis of 3,660 calving records showed that the average economic loss due to uterine inflammations was EUR 122.8 per case, with an increased number of open days being the predominant source of loss, accounting for 57.6% of the total loss (Kern et al., 2018).

The aim of this study was to demonstrate the possible role of uterine infections caused by different bacteria and viruses in the infertility of dairy cattle.

Materials and methods

Animals

The samples were collected from 40 infertile dairy cows kept at five dairy farms in March 2021 (herd size: 650–1,800 cows; milk production: 9,500–11,600 kg/cow/year). At these farms, the culling rate for slaughter ranged between 32% and 40% in the previous year. On average, 40% of the total culling rate was associated with infertility (ranges: 32–48%). All cows were Holstein-Friesian, fed a total mixed ration (TMR) and bred by artificial insemination (AI). The cows included in this study exceeded 220 days in milk (DIM), had been inseminated at least three times, and had not become pregnant.

Uterine swab collection and bacteriology ($n = 40$)

After cleaning the vulva and the surrounding region with clean water, uterine swab samples were collected using swabs protected from vaginal contamination (Equivet uterine swab, Kruuse, Marslev, Denmark). All swab samples were transported to the laboratory in a refrigerator at 4°C within 2-4 hours of sample collection. Swabs of uterine contents were inoculated onto blood agar, MacConkey agar and Sabouraud Dextrose agar incubated aerobically, blood agar incubated anaerobically, *Campylobacter*-selective agar (Skirrow's medium) incubated under microaerophilic conditions, and chocolate agar incubated in 10% CO_2 at 37°C for up to 3 days. Bacterial and fungal isolates were identified to the genus level on the basis of cultural, morphological and biochemical features, and to the species level using the MALDI-TOF system.

Isolation of *Ureaplasma* and *Mycoplasma* species was also attempted in Ureaplasma medium (Mycoplasma Experience Ltd., UK) and in Mycoplasma

broth medium (pH 7.8) [Thermo Fisher Scientific Inc., (Oxoid Inc.), Waltham, MA] supplemented with 0.5% (w/v) sodium pyruvate, 0.5% (w/v) glucose and 0.005% (w/v) phenol red. Additional swabs were taken for *Chlamydiales, C. burnetii, U. diversum* and *Mycoplasma* species-specific PCR tests. The DNA was extracted from the samples with the NucleoSpin Tissue Mini kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), according to the manufacturer's instructions. A species-specific PCR assay targeting the 16S rRNA region was used to detect the presence of *U. diversum* in the samples (Tramuta et al., 2011). A genus-specific PCR assay targeting the 16S/23S rRNA intergenic spacer region was used to detect the presence of *Mycoplasma* species in the samples (Lauerman et al., 1995). Members of the *Chlamydiales* order were detected with the 16S rRNA gene-based *Chlamydiales*-specific qPCR assay as described previously (Lienard et al., 2011). Real-time PCR assay specific for the IS1111 element was used to detect the presence of *C. burnetii* in the uterine swabs. The sixth samples were tested by real-time PCR for BVDV and BoHV-1 DNA by the Bio-T kit® BVDV & BHV1-gE PCR kit (Biosellal, Dardilly, France) according to the manufacturer's instructions. The detected bacteria were categorised (Table 1) according to their pathogenic potential reported in previous studies (Williams et al., 2007; Borel et al., 2018; De Biase et al., 2018; Appiah et al., 2020; Santos et al., 2021).

The category of uterine pathogens included bacterial species reported to be associated with uterine lesions ('recognised uterine pathogens'), the category of potential bacterial pathogens included species frequently isolated from the uterus of cows presenting endometritis and commonly associated with uterine lesions, while the category of opportunistic pathogens included bacterial species occasionally isolated from the uterine lumen and not associated with endometritis (contaminants).

Blood samples (n = 40)

Blood samples were collected at same time as swab samples from all the 40 cows studied and were examined by ELISA. Commercial ELISA kits (ID Screen® Q Fever Indirect Multispecies, IDVet Inc., Grabels, France) were used according to the manufacturer's instructions. The serum samples were examined by two different complement fixation tests (CFT) utilising *C. burnetii* phase I and II antigens, according to the manufacturer's instructions (Virion/Serion GmbH, Würzburg, Germany), and the Manual

Table 1. Categorisation of bacteria isolated by aerobic and anaerobic culture or detected by PCR (*Coxiella burnetii, Chlamydiales, Mycoplasma* and *Ureaplasma*) from 40 uterine swabs, according to their expected pathogenic potential in the uterus (Williams et al., 2007; Borel et al., 2018; De Biase et al., 2018; Appiah et al., 2020; Santos et al., 2021)

Recognised uterine pathogens	Potential uterine pathogens	Opportunistic uterine contaminants
Escherichia coli (2)	*Bacillus licheniformis* (1)	*Clostridium perfringens* (1)
Coxiella burnetii (3)	Non-haemolytic streptococci (5)	*Staphylococcus* species, coagulase-negative (3)
Mycoplasma spp. (2)	*Histophilus somni* (2)	*Bacillus* spp. (10)
Ureaplasma spp. (3)	*Chlamydiales* (22)	*Corynebacterium* spp. (4)
	Candida krusei (2)	

of Diagnostic Tests and Vaccines for Terrestrial Animals (World Organisation for Animal Health, 2018).

Histological examination of uterine biopsy samples (*n* = 40)

A previously disinfected biopsy apparatus (Kruuse, Marslev, Denmark) was introduced into the uterus, and an approx. 0.5 × 0.5 × 1 cm portion of the uterine mucosa was chipped off from the dorsal wall of the uterine body, at the junction of the uterine horns, under control by rectal palpation. All biopsies were performed by the same operator. The biopsy samples were fixed in 10% buffered formaldehyde solution for 24 h. Subsequently the samples were embedded in paraffin, cut into 4 µm thick sections, which were then stained with haematoxylin and eosin. The samples were evaluated on the basis of the criteria described by Chapwanya et al. (2009).

Statistical method

Histological findings were classified as either mild (category 1) or severe (category 2 or 3). The bacteriological results of the samples were categorised according to uterine pathogenicity (recognised, potential, opportunistic, and no uterine pathogens). The samples were allocated to these four categories based on the bacterium with the highest expected uterine pathogenic potential (e.g., if both a recognised and a potential uterine pathogen was found in the sample, it was classified as recognised). The relationship between severity class (mild or severe) and uterine pathogenicity was examined by mixed effects logistic regression with farm as the random effect. Model building was performed using the glmmTMB package in R (Brooks et al., 2017). Statistical analyses were performed in R version 4.0.5 (R Core Team, 2021). The level of significance was set to 0.05.

Results

Bacteriology and virology

The results of bacteriological and virological examinations are summarised in Table 1. Eight samples showed mixed infections and the remaining specimens yielded facultative anaerobic bacteria in pure culture. The mixed cultures contained mainly *Bacillus* spp. and *Streptococcus* spp. The most common facultative anaerobic pathogens were *Streptococcus* spp., *Staphylococcus* spp. and *Corynebacterium* spp. We found *Escherichia coli* as a recognised uterine pathogen in two cases. Potential uterine pathogens were *Bacillus licheniformis* (one case), non-haemolytic streptococci (five cases), *Histophilus somni* (two cases) and *Candida krusei* (two cases). Opportunistic uterine contaminants included *Clostridium perfringens* (one case), *Staphylococcus* species, coagulase-negative (three cases), *Bacillus* spp. (ten cases), and *Corynebacterium* (four cases). *Chlamydiaceae* DNA was detected by real-time PCR in 22/40 samples (55%). We detected *C. burnetii* DNA in 3/40 cases (7.5%) by real-time PCR. *Mycoplasma* and *Ureaplasma* DNA were found in 2/40 (5%) and 3/40 (7.5%) samples, respectively. BVDV and BoHV-1 DNA was not detected in any samples. ELISA testing showed 26/40 (65%) individual seropositivity among the cows examined, while 7/40 (17.5%) and 6/40 (15%) of the cows exhibited low titres (1:10–1:40) by Phase I and Phase II CFT, respectively. One blood sample 1/40 (2.5%) showed high titres (1:640) by Phase II CFT.

Histological examination of the uterine biopsy samples

Three of the uterine biopsy samples were not suitable for evaluation due to a lack of endometrial mucosa. Twenty-two cases (59%) showed the presence of mild lympho-histiocytic infiltration in the mucosa, and were included in category 1

(Fig. 1a). Prominent leukostasis was also evident in two of these cases (Fig. 1b). Moderate lympho-histiocytic infiltration of the endometrium was evident in 13 cases (35%), which were included in category 2 (Fig. 2). The remaining two cases were included in category 3, as they presented severe inflammatory cell infiltration in the endometrium, including lympho-histiocytes and neutrophil granulocytes (Fig. 3).

The number of samples in the recognised, potential, opportunistic, and no uterine pathogen categories based on the highest expected uterine pathogenic potential was 7 (17.5%), 22 (55.0%), 6 (15.0%), and 5 (12.5%), respectively. Severe histological lesions occurred in 28.6% (2/7), 40.0% (8/20), 50.0% (3/6), and 50.0% (2/4) of the samples in the recognised, potential, opportunistic, and no uterine pathogen categories, respectively. No relationship was found between the severity of histological lesions and uterine pathogenicity ($P = 0.8555$).
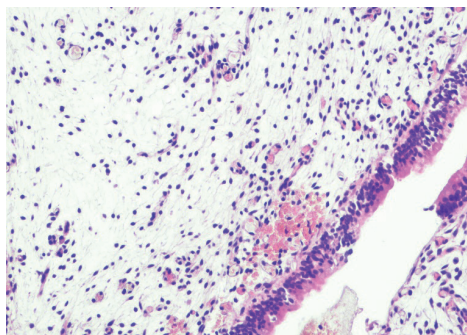

Fig. 1a. Category 1; focal haemorrhage, oedema and mild lympho-histiocytic infiltration in the stratum compactum and stratum spongiosum of the endometrium. Cattle, haematoxylin and eosin (HE), ×200
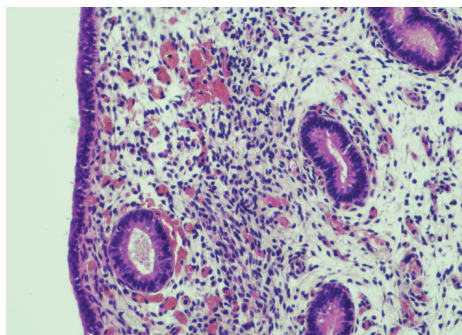

Fig. 2. Category 2; focal haemorrhage, oedema and moderate lympho-histiocytic infiltration in the stratum compactum and stratum spongiosum of the endometrium. Cattle, HE, ×200
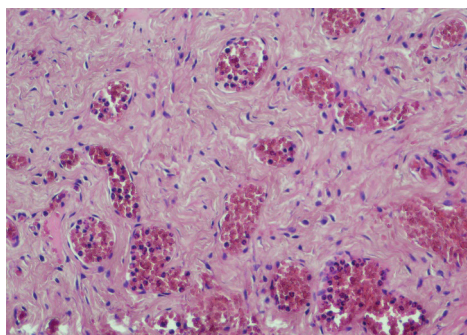

Fig. 1b. Pronounced leukostasis in the stratum spongiosum in a case included in Category 1. Cattle, HE, ×200
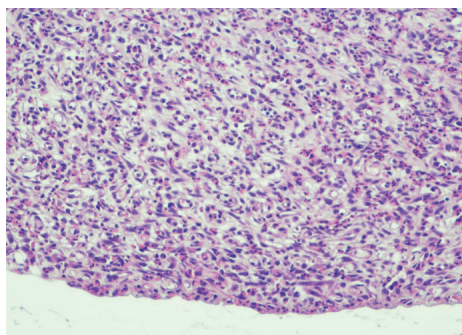

Fig. 3. Category 3; moderate lympho-histiocytic and severe neutrophil granulocytic infiltration in the stratum compactum and stratum spongiosum of the endometrium. The epithelial layer of the endometrium is detached. Cattle, HE, ×200

Discussion

Unfortunately, there is little information on the bacterial causes of infertility in cows. *Campylobacter fetus* ssp. *venerealis, Leptospira, Mycoplasma, Ureaplasma, Chlamydia, Histophilus somni* and *C. burnetii* have all been associated with bovine infertility, but at present the relative prevalence and importance of these agents are unknown (Yaeger and Holler, 2007; Agerholm, 2013). The most frequently isolated bacteria were *Bacillus* spp. and *Streptococcus* spp., either in pure culture or in mixed cultures with other microorganisms, and they represented 56.9% of all isolates in this study. Although *Bacillus* spp. are opportunistic microorganisms, they can occasionally contribute to the development of bovine abortion (Yaeger and Holler, 2007). In one case, we found *Bacillus licheniformis*, a potential abortifacient bacterium (Agerholm et al., 1999). The isolated *Staphylococcus* spp. and *Streptococcus* spp. are part of the normal microflora of mucosal surfaces. We detected *Escherichia coli* in two cases. This bacterium was cultured some days after parturition, but around breeding time, *E. coli* can cause endometritis and is a well-recognised uterine pathogen (Brodzki et al., 2014). *Histophilus somni* was found in two cases. This bacterium is known to cause diseases of the reproductive tract in cattle but the virulence of the strains may vary, the cause of which has not been defined (Pérez et al., 2010). The *Corynebacterium* spp. and *Staphylococcus* spp. found in this study generally constitute additional flora along with the major uterine pathogens (Watts et al., 2000; Ghanem et al., 2015). The isolation of *Mycoplasma* spp. and *Ureaplasma* spp. has been linked with reproductive disorders in cattle. Several studies have indicated an association between the occurrence of Mollicutes infection and bovine infertility (Pfützner

and Sachse, 1996; Macedo et al., 2018; Santos et al., 2021). We confirmed five cases of Mollicutes infection by mPCR and attempted to recover the pathogens by microbial culture but no growth was obtained. *Chlamydiaceae* DNA was detected by real-time PCR in 22/40 (55%) samples, thus *Chlamydiaceae* were the most frequently found pathogens in this study. *Chlamydia abortus* is incriminated as a cause of bovine endometritis with resulting infertility, while other *Chlamydiales* are associated with reproductive problems though further research is needed to reveal their exact aetiological role (Wittenbrink et al., 1993; Borel et al., 2018). *Coxiella burnetii* was demonstrated in the macrophages of the endometrium in cattle (De Biase et al., 2018). This study found mild to severe chronic endometritis in PCR-positive animals. We detected three *C. burnetii* PCR-positive cases by real-time PCR assay. However, only one of the three PCR-positive cases tested positive by ELISA of the blood as well, which is in agreement with the findings reported by Guatteo et al. (2006). Serological data and PCR detection of the pathogen in the uterus may not be correlated. We found diffuse endometritis in two cases, but no correlation between this agent and endometritis. Our previous research found 48.2% *C. burnetii* seropositivity by ELISA among pregnant cows, while the seropositivity of animals that had lost their pregnancy at an early stage was 80.5%. This study found a higher seropositivity rate (65%) in the infertile cows compared with the pregnant animals in the previous study. We detected only a single case with 1:80 titre by CFT (Phase II), which is indicate of an active phase of *C. burnetii* infection (Dobos et al., 2020). *Clostridium* spp. was detected in one case. Moderate endometritis was evident in 13 cases and we also detected two recognised uterine pathogens (*E. coli* and *Bacillus licheniformis)* in these

cases. *Chlamydiaceae* DNA was detected in 22 cases. Three of these 22 cases were also positive for *C. burnetii* by PCR. Ten (45.4%) of the 22 cases showed diffuse and moderate endometritis (Category 2 or 3) while the rest of cases were mild (Category 1). *Chlamydia* spp. were identified in 22/40 cases (55%) in this study, which is higher than the 12.9% value obtained in a previous research (Fábián et al., 2007). We did not find a correlation between these agents and endometritis in this study. There was no correlation between *Mycoplasma* and *Ureaplasma* PCR positivity and the histological or bacteriological findings. All the farms included in the study practised vaccination against infectious bovine rhinotracheitis (IBR), which was possibly the reason why BoHV-1 DNA was not detected in any samples. However, as three of the five farms were infected with BVDV and none of the five farms were vaccinated against BVD, it was surprising that BVDV DNA was not detected by PCR.

In conclusion, the presence of pathogenic agents in the uterus evidently affects the chances of embryo survival (Sheldon et al., 2006). A healthy uterus and endometrium are key elements for embryo implantation. Interactions between the endometrium and the conceptus are influenced by many factors mostly related to the uterine environment. Pathogenic and potentially pathogenic bacteria often persist for long periods of time, causing uterine disease and changing the uterine environment, which are the key causes of infertility in cattle. This study highlights the need for a better understanding of the aetiology and pathogenesis of bovine infertility and the possible causative agents. As reproductive performance is a major factor influencing the profitability of dairy farms, further investigations into the possible causative agents and factors of infertility are needed.

References

1. AGERHOLM, J. S. (2013): *Coxiella burnetii* associated reproductive disorders in domestic animals – a critical review. Acta Vet. Scand. 55, 13.
2. AGERHOLM, J. S., N. E. JENSEN, V. DANTZER, H. E. JENSEN and F. M. AARESTRUP (1999): Experimental infection of pregnant cows with *Bacillus licheniformis* bacteria. Vet. Pathol. 36, 191-201.
3. APPIAH, M. O., J. WANG and W. LU (2020): Microflora in the reproductive tract of cattle: A review. Agriculture 10, 232.
4. BENIĆ, M., N. MAĆEŠIĆ, L. CVETNIĆ, B. HABRUN, Ž. CVETNIĆ, R. TURK, D. ĐURIČIĆ, M. LOJKIĆ, V. DOBRANIĆ, H. VALPOTIĆ, J. GRIZELJ, D. GRAČNER, J. GRBAVAC and M. SAMARDŽIJA (2018): Bovine mastitis: a persistent and evolving problem requiring novel approaches for its control - a review. Vet. arhiv 88, 535-557.
5. BONNEVILLE-HÉBERT, A., E. BOUCHARD, D. D. TREMBLAY and R. LEFEBVRE (2011): Effect of reproductive disorders and parity on repeat breeder status and culling of dairy cows in Quebec. Can. J. Vet. Res. 75, 147-151.
6. BOREL, N., A. POLKINGHORNE and A. POSPISCHIL (2018): A review on chlamydial diseases in animals: still a challenge for pathologists? Vet. Pathol. 55, 374-390.
7. BRODZKI, P., M. BOCHNIARZ, A. BRODZKI, Z. WRONA and W. WAWRON (2014): *Trueperella pyogenes* and *Escherichia coli* as an etiological factor of endometritis in cows and the susceptibility of these bacteria to selected antibiotics. Polish J. Vet. Sci. 17, 657-664.
8. BROOKS, M. E., K. KRISTENSEN, K. J. VAN BENTHEM, A. MAGNUSSON, C. W. BERG, A. NIELSEN, H. J. SKAUG, M. MAECHLER and B. M. BOLKER (2017): glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9, 378-400.
9. BUROVIĆ, J. (2020): Isolation of bovine clinical mastitis bacterial pathogens and their antimicrobial susceptibility in the Zenica region in 2017. Vet. stn. 51, 47-52. (In Croatian).
10. CABRERA, V. E. (2014): Economics of fertility in high-yielding dairy cows on confined TMR systems. Animal 8, 211-221.
11. CAN, M. F., V. S. ATASEVEN and C. YALÇIN (2016): Estimation of production and reproductive performance losses in dairy cattle due to bovine herpesvirus 1 (BoHV-1) infection. Vet. arhiv 86, 499-513.
12. CHAPWANYA, A., K. G. MEADE, M. L. DOHERTY, J. J. CALLANAN, J. F. MEE and C. O'FARRELLY (2009): Histopathological and molecular evaluation of Holstein-Friesian cows postpartum: toward an improved understanding of uterine innate immunity. Theriogenology 71, 1396-1407.
13. DE BIASE, D., A. COSTAGLIOLA, F. DEL PIERO, R. DI PALO, D. CORONATI, G. GALIERO, B. D. UBERTI, M. G. LUCIBELLI, A. FABBIANO, B.

DAVOUST, D. RAOULT and O. PACIELLO (2018): *Coxiella burnetii* in infertile dairy cattle with chronic endometritis. Vet. Pathol. 55, 539-542.

14. DOBOS, A., G. GÁBOR, E. WEHMANN, B. DÉNES, B. PÓTH-SZEBENYI, Á. B. KOVÁCS and M. GYURANECZ (2020): Serological screening for *Coxiella burnetii* in the context of early pregnancy loss in dairy cows. Acta Vet. Hung. 68, 305-309.

15. DE VRIES, A. (2006): Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89, 3876-3885.

16. FÁBIÁN, K., L. MAKRAI, K. SACHSE, L. SZEREDI and L. EGYED (2008): An investigation of the aetiological role of bovine herpesvirus 4 in bovine endometritis. Vet. J. 177, 289-292.

17. FOLNOŽIĆ, I., R. TURK, D. ĐURIČIĆ, S. VINCE, J. PLEADIN, Z. FLEGAR-MEŠTRIĆ, H. VALPOTIĆ, T. DOBRANIĆ, D. GRAČNER, M. SAMARDŽIJA (2015): Influence of Body Condition on Serum Metabolic Indicators of Lipid Mobilization and Oxidative Stress in Dairy Cows During the Transition Period. Reprod. Domest. Anim. 50, 910-917.

18. FOLNOŽIĆ, I., M. SAMARDŽIJA, D. ĐURIČIĆ, S. VINCE, S. PERKOV, S. JELUŠIĆ, H. VALPOTIĆ, B. BEER LJUBIĆ, M. LOJKIĆ, D. GRAČNER et al. (2019): Effects of in-feed clinoptilolite treatment on serum metabolic and antioxidative biomarkers and acute phase response in dairy cows during pregnancy and early lactation. Res. Vet. Sci. 127, 57-64.

19. GHANEM, M. E., E. TEZUKA, B. DEVKOTA, Y. IZAIKE and T. OSAWA (2015): Persistence of uterine bacterial infection, and its associations with endometritis and ovarian function in postpartum dairy cows. J. Reprod. Dev. 61, 54-60.

20. GUATTEO, R., F. BEAUDEAU, M. BERRI, A. RODOLAKIS, A. JOLY and H. SEEGERS (2006): Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control. Vet. Res. 37, 827-833.

21. KERN, L., I. FODOR, O. G. VARGA-BALOGH, L. ÓZSVÁRI and GY. GÁBOR (2018): The impact of postpartum uterine diseases on reproductive performance and their economic losses on large Hungarian dairy farms. Magy. Allatorvosok 140, 717-726. (In Hungarian).

22. KOVÁCS, L., L. RÓZSA, M. PÁLFFY, P. HEJEL, W. BAUMGARTNER and O. SZENCI (2020): Subacute ruminal acidosis in dairy cows – physiological background, risk factors and diagnostic methods. Vet. stn. 51, 5-17.

23. LAUERMAN, L. H., A. R. CHILINA, J. A. CLOSSER and D. JOHANSEN (1995): Avian mycoplasma identification using polymerase chain reaction amplicon and restriction fragment length polymorphism analysis. Avian Dis. 39, 804-811.

24. LIENARD, J., A. CROXATTO, S. AEBY, K. JATON, K. POSFAY-BARBE, A. GERVAIX, and G. GREUB (2011): Development of a new Chlamydiales-specific real-time PCR and its application to respiratory clinical samples. J. Clin. Microbiol. 49, 2637-2642.

25. MACÊDO, A. A. M., J. M. B. OLIVEIRA, B. P. SILVA, J. M. BORGES, L. B. F. SOARES, G. M. SILVA, S. B. SANTOS, R. A. MOTA and J. W. PINHEIRO-JÚNIOR (2018): Occurrence of *Mycoplasma bovigenitalium* and *Ureaplasma diversum* in dairy cattle from to Pernambuco state, Brazil. Arq. Bras. Med. Vet. Zootec. 70, 1798-1806.

26. MEADOWS, C., P. J. RAJALA-SCHULTZ and G. S. FRAZER (2005): A spreadsheet-based model demonstrating the nonuniform economic effects of varying reproductive performance in Ohio dairy herds. J. Dairy Sci. 88, 1244-1254.

27. ÓZSVÁRI, L. and J. KERÉNYI (2004): Quantification of losses due to reproductive disorders on a large-scale Holstein-Friesian dairy farm. Magy. Allatorvosok 126, 523-531. (In Hungarian).

28. PÉREZ, D. S., F. A. PÉREZ and G. BRETSCHNEIDER (2010): *Histophilus somni*: pathogenicity in cattle. An update. An. Vet. Murcia 26, 5-21.

29. PFÜTZNER, H. and K. SACHSE (1996): *Mycoplasma bovis* as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. Rev. Sci. Tech. (International Office of Epizootics) 15, 1477-1494.

30. R Core Team (2021): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

31. SANTOS, M. N. JR., N. S. DE MACÊDO NERES, G. B. CAMPOS, B. L. BASTOS, J. TIMENETSKY and L. M. MARQUES (2021): A review of *Ureaplasma diversum*: A representative of the *Mollicute* class associated with reproductive and respiratory disorders in cattle. Front. Vet. Sci. 8, 572171.

32. SHELDON, I. M., G. S. LEWIS, S. LEBLANC and R. O. GILBERT (2006): Defining postpartum uterine disease in cattle. Theriogenology 65, 1516-1530.

33. TRAMUTA, C., D. LACERENZA, S. ZOPPI, M. GORIA, A. DONDO, A. FERROGLIO, P. NEBBIA and S. ROSATI (2011): Development of a set of multiplex standard polymerase chain reaction assays for the identification of infectious agents from aborted bovine clinical samples. J. Vet. Diagn. Invest. 23, 657-664.

34. WATHES, D. C., C. F. OGUEJIOFOR, C. THOMAS and Z. CHENG (2020): Importance of viral disease in dairy cow fertility. Engineering 6, 26-33.

35. WATTS, J. L., D. E. LOWERY, J. F. TEEL and S. ROSSBACH (2000): Identification of *Corynebacterium bovis* and other coryneforms isolated from bovine mammary glands. J. Dairy Sci. 83, 2373-2379.

36. WILLIAMS, E. J., D. P. FISCHER, D. E. NOAKES, G. C. W. ENGLAND, A. RYCROFT, H. DOBSON and I. M. SHELDON (2007): The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68, 549-559.

37. WITTENBRINK, M. M., H. A. SCHOON, D. SCHOON, R. MANSFELD and W. BISPING (1993): Endometritis in cattle experimentally induced by *Chlamydia psittaci*. J. Vet. Med. B 40, 437-450.

38. World Organisation for Animal Health (OIE) (2018): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 8th edition. OIE, Paris.

39. YAEGER, M. J. and L. D. HOLLER (2007): Bacterial causes of bovine infertility and abortion. In: Youngquist, R. S. and Threlfall, W. R. (eds). Current Therapy in Large Animal Theriogenology. Elsevier, St. Louis, MO, USA. Pp. 389-399.

40. YOO, H. S. (2010): Infectious causes of reproductive disorders in cattle. J. Reprod. Dev. 56, 53-60.

Neplodnost u mliječnih krava – mogući bakterijski ili virusni uzroci

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U ovom su istraživanju prikupljeni brisevi maternice i biopsijski uzorci 40 neplodnih mliječnih krava s pet mliječnih farmi u Mađarskoj. Uzorci su testirani na bakterije, uključujući; *Coxiella burnetii*, klamidiju, mikoplazmu i ureaplazmu te na viruse uključujući goveđi herpesvirus 1 (BoHV-1) i virus virsnog proljeva goveda (BVDV). DNK *Chlamydiaceae* otkriven je PCR testom u stvarnom vremenu u 22/40 (55 %) uzoraka. DNK bakterije *Coxiella burnetii* otkriven je u 3/40 (7,5 %) slučajeva PCR testom u stvarnom vremenu. DNK mikoplazme i ureaplazme pronađen je u 2/40 (5 %), odnosno 4/40 (10 %) krava. DNK virusa BVD i BoHV-1 niti u jednom uzorku nije otkriven. *Escherichia coli* kao priznati maternični patogen pronađen je u dva slučaja. Pronađeni su sljedeći potencijalni maternični patogeni: *Bacillus licheniformis* (jedan slučaj), nehemolitički streptokoki (pet slučajeva), *Histophilus somni* (dva slučaja) i *Candida krusei* (dva slučaja). Uzorci krvi su istovremeno prikupljeni kad i brisevi od svih 40 pokusnih krava. Njihova pretraga na protututijela *C. burnetii* ELISA metodom otkrila je seropozitivnost u 26/40 krava (65 %). Histološka pretraga uzoraka biopsije maternice pokazala je prisutnost blage limfohistiocitotske infiltracije u sluznici u 22 slučaja (59 %). Umjerena limfohistiocitotska infiltracija endometrija bila je prisutna u 13 slučajeva *(35 %)*, dok je u dva slučaja (6 %) otkrivena ozbiljna upalna stanična infiltracija endometrija s limfohistiocitima i neutrofilnim granulocitima. Premda nije bilo moguće dokazati statističku korelaciju između ozbiljnosti histoloških lezija endometrija i maternične patogenosti bakterija (*P* = 0,8555), endometrioza određenog stupnja ozbiljnost i/ili priznati ili potencijalni maternični patogeni pronađeni su u svim uzorcima. Ovi posljednji mogu prouzročiti razvoj neplodnosti bilo skupno ili pojedinačno.

Ključne riječi: *neplodnost goveda, mliječna krava, histološka pretraga, biopsija maternice, bris maternice*