Original Paper

The prophylaxis of puerperal infections of cows' genitals by *Lactobacillus* spp.

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Many bacterial species are currently known as etiologic agents of dairy cattle metritis. Antimicrobials for endometritis treatment are infused into the uterus, and these therapies are aimed to achieve high concentrations of antibiotics in the site of infection. The aim of this work was to verify the possibility of using a non-antibiotic active substance to prevent the emergence of postpartum infections in cows. The study was realised in 12 herds of Holstein, Simental and Pinzgau cattle (4,563 animals), where the incidence of postpartum reproductive disorders was higher than 20%. After parturition, animals (4,563) were divided into four groups: (I) 1,967 primipara cows treated, (II) 2,596 pluripara cows treated, (III) 638 primipara cows untreated and (IV) 667 pluripara cows untreated. Animals were treated 24 hours after parturition with 100 ml *Lactobacillus* spp. (LBC), lyophilised preparation (1 ml contained 1.0 $\times 10^9$ microorganisms). Lyophilisate was diluted just before the application with a sterile solvent with pH 4.4–4.7. The most prevalent pathogens were *E. coli* (74.7%) and *Trueperella pyogenes* (17.3%). *E. coli* caused up to 88.2% of genital inflammation – mostly endometritis. The positive effect of the preventive application of preparation based on LBC significantly eliminated the occurrence of bacterial microflora after the parturition on the 14th and 20th day of the puerperium, respectively. The preventive application was manifested to a demonstrable extent also in the subsequent reproductive parameters of cows. The preparation appeared to be more effective in primipara, especially concerning the incidence of inflammatory conditions of the genitals (P <0.01).

Keywords: cows, puerperium, prevention, *Lactobacillus* spp.

1 Introduction

Foetal membranes are released after calving, ischemic necrosis develops and several layers of caruncular epithelium separate, surface defects are restored with a new epithelium (Roberts, 1971), and myometrial muscle fibres are shortened. Most of these changes occur early in the postpartum period (first two weeks) before the pituitary-ovarian axis induces return of the oestrous cycle. Most dairy cows will have their first postpartum ovulation in about 17 to 27 days after calving (Stevenson, 1997). Metritis affects up to 40% of dairy cows and it is usually treated with antibiotics. In spite of their advantages, there is an increased concern about antibiotic resistance leading to the research of alternative methods (Genís et al., 2018) The normal involution process produces a large volume of necrotic tissue in the form of lochia, and significant bacterial contamination of the postpartum uterine cavity is common. Abundant evidence proves that the vast majority of cows develop uterine bacterial contamination after 2 to 3 weeks after calving (Sheldon, 2004), including bacteria associated with the uterine disease. Up to 90% of recently calved cows have uterine infections 1 to 2 week after parturition. Infection and inflammation of the uterus and cervix affect approximately one out of every three dairy cows with substantial impacts on the probability and timing of pregnancy (LeBlanc et al., 2011; LeBlanc, 2014; Bicalho et al., 2012). Cows with retained placenta and labour complications show increased bacterial contamination of the uterus (Dohmen et al., 2000).

Bacterial uterine infections not only disrupt the function of the uterus, but also affect the ovaries as well as hypothalamic and hypophyseal control centres. The uterine lumen of most postpartum cows is contaminated with environmental bacteria. Elimination of contamination depends on the level of the uterine involution, regeneration of endometrium and uterine

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protective mechanisms. The flora cultured in the early postpartum period represents a broad spectrum of environmental contaminants (e.g., *Escherichia coli*, *Actinomyces pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Streptococcus* spp., and *Pasteurella multocida*) and includes some anaerobic species (e.g., *Clostridium* spp., *Bacterioides* spp., and *Fusobacterium* spp.) (Williams et al., 2005; Noakes et al., 1991; Miller et al., 2007; Westerman et al., 2010; Sens and Heuwieser, 2013). The most prevalent pathogens are *Escherichia coli* (37% of isolated pathogenic bacteria) and *A. pyogenes* (49%) (Williams et al., 2005). *E. coli* still paves the way for the action of *A. pyogenes* (Wiliams et al., 2007). *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Bacteroides* spp. are most commonly active in the early puerperium period in the first week after birth (Dohmen et al., 1995; Dohmen et al., 2000; Bicalho et al., 2012). The defence mechanism of the uterus is responsible for fighting the bacterial contamination of the uterus via a range of anatomical, physiological, phagocytic and inflammatory mechanisms. The first and most critical phagocytic cells entering the lumen are neutrophils. Functional capacity of neutrophils in many postpartum cows is reduced. Zerbe et al. (2000) demonstrated that metabolic disease and especially increased blood level of liver triacylglycerols were associated with reduced cytotoxic activity in neutrophils from the general circulation and the uterine wall, predisposing to uterine disease. Lewis (2003) showed that luteal phase concentrations of progesterone suppress the immune response, making the uterus more susceptible to bacterial infection. The diagnostic process in acute forms of the endometrium is not difficult. Still, with regards to the long-time treatment, there is a loss of proper reproduction parameters and complete loss of milk production during the antibiotic treatment due to possible antibiotic residues in milk. Reducing a load of potential bacterial pathogens in the uterus immediately after calving could reduce the risk of uterine disease (LeBlanc et al., 2011). Traditional antimicrobial treatments against metritis are not very effective as only 67–77% of treated cows decrease fever after 5–10 days of treatment and there is not always resolution of the fetid odor (LeBlanc, 2008). Furthermore, an improvement of the reproductive performance of the treated animals is not always found, especially in cases of sustained inflammation LeBlanc, 2008). The recommendations from the World Health Organization (Deng et al., 2015) to reduce antibiotic treatments leads to investigate alternative therapies. For this reason and often for the reason of existing resistance, scientists are already looking for a long time for antibiotics substitutions with other sufficiently effective preparations (McDougall et al., 2013). These preparations could fulfil current ecological criteria as well as not being a risk for the possible onset of resistance in children. Testing of alternative matters was focused on immunostimulating effects of some microbial cultures, which should affect similarly as enteral infections (Saxelin, 1997) and to endometrium either (Kummer et al., 1997; Husain and Daniel, 1992). Protective effects of *Lactobacillus* spp. as natural commensals of the vaginal environment in humans are known already for a long time. They support not only physiological biocenosis but also protect vaginal secretion mechanisms (Williams et al.,1988, Corsetti et al., 2005). There are known immunomodulatory effects and inhibition effects of some *Lactobacillus* spp. strain to the pathogen microflora of endometrium. Therefore the aim was to verify the possibility of using a non-antibiotic active substance to prevent the emergence of postpartum infections in cows

2 Material and methods

2.1 Animals

This study was realised over 8 years in 12 herds of Holstein (4,071), Simental (375) and Pinzgau (117) cattle where the incidence of postpartum reproductive disorders was higher than 20%, conception rate after parturition was lower than 45% and average days open was 145 days (131 to 162 days). After parturition, animals (4,563) were divided into four groups: (I) 1,967 primipara cows treated, (II) 2,596 pluripara cows treated and (III) 638 primipara cows untreated, (IV) 667 pluripara cows untreated (Table 1).

2.2 Preparation and application

Animals were treated 24 h after parturition with 100 ml *Lactobacillus* spp. (LBC), lyophilised preparation (1cm^3 contained 1.0 $\times 10^9$ microorganisms). Lyophilisate was diluted just before the application with a sterile solvent with pH 4.4–4.7. Preparation was applied with PVC catheter and Jannete type 150 ccm syringe (Ivanics, Hungary) intrauterine and intravaginal (50 ml and 50 ml resp.). Before and after the LBC application, samples from the uterus and vagina were taken for bacteriological examination by a standard procedure (Šťastná et al., 2007). The vaginal and cervical swabs for microbiological examination were taken for sterile dry swabs (Stirilab) using a vaginal speculum. They were transported and processed to the laboratory within 3 hours of collection. The samples were transferred to the blood agar (BioLab Slovakia). Selective culture media (BioLab Slovakia) – Enda soil (*Escherichia coli* and *Treuperella pyogenes*), Chocolate agar 10% CO_2 (*Histophilus somni*) and Blood agar (*Clostridium* spp.) were used for selective cultivation. No significant differences between breeds were found in any of the monitored parameters; therefore, the data were processed and evaluated comprehensively for the

Table 1 Number and distribution of animals

Farm	% EM	Treated	Untreated	Treated	Untreated
		Primipara		Pluripara	
1	22.8	61	54	77	55
2	21.6	57	50	78	55
3	23.4	15	25	52	50
4	26.1	83	56	97	55
5	22.7	32	26	58	54
6	22.3	877	157	978	122
7	24.8	102	57	399	59
8	23.4	426	56	520	54
9	27.1	181	52	160	55
10	22.4	13	26	47	28
11	24.6	98	54	102	54
12	25.5	22	25	28	26
All cows 4,563	1,967	638	2,596	667	

EM endometritis

group (primipara and pluripara – cows). The obtained data were statistically evaluated by software analysis using Statgraphic 18 data.

3 Results and discussion

Postpartum metritis is one of the most important disorders in cattle (Foldi et al., 2006) causing high economic losses due to prolonged days open and prolonged calving period. The uterine disease is associated with a lower conception rate, increased intervals from calving to conception and more cattle culled for failure to conceive (LeBlanc et al., 2001). The prevalence rate of postpartum infections, mostly endometritis, depends on the breeding level. In the monitored group of 12 breeds (4,563 animals) the occurrence of postpartum inflammatory incidents (23.89%; from 21.6% to 27.1%, Table 1) caused an increase in days open to average 145 days. The superficial epithelium of the uterus is disrupted after birth and contact with fluid and tissue residues can promote bacterial growth (Azawi, 2008). The postpartum activity of the uterus helps to eliminate these residues from the endometrium in the shortest possible time by elimination of the lochia and involution of the uterus. The physiological course of the cow parturition and the postpartum period provides a presumption for rapid cleansing of the uterus, its early involution and early onset of a new postpartum oestrous cycle.

Table 2 Reproduction parameters of cows and occurrence of genitalia infections after preventive treatment

		Treated		Untreated		Tt T : U
		$x \pm s$	v	$x \pm s$	v	
C–E	Primipara	21.66±1.347	1.00	24.18±0,658	2.72	P <0.01
	Pluripara	21.39±1.714	8,1	23.33±0.592	2.53	
C-1st E	Primipara	55.32±3.041	5.49	57.77±3.893	6.73	
	Pluripara	53.34±3.068	5.65	55.39±3.432	6.19	
DO	Primipara	97.51±4.977	5,1	119.56±5.276	4.41	P <0.01
	Pluripara	96.15±5.532	5.75	115.28±3.894	3.38	P <0.01
% + G	Primipara	53.22±3.903	7.33	43.63±2.723	6.24	P <0.01
	Pluripara	55.08±3.447	6.26	47.48±2.385	05.2	P <0.01
%GI	Primipara	3.68±0.428	11.59	1.58±2.744	17.6	P <0.01
	Pluripara	2.89±0.871	30.13	14.18±2.098	20.5	P <0.01

C–E calving to estrous (days), C-1st S calving to 1st AI (days), DO days open, G + pregnancy (%), GI genitals inflammations (%), Tt T : U – *t*-test treated : untreated

Table 3 Occurrence puerperal microflora in genitalia of cows before and after preventive treatment (n = 4,563)

Microorganism	1st day post-calving	12th day post-calving			
	%	treated (%)		untreated (%)	
		NP	GI	NP	GI
Escherichia coli	74.7	3.7	10.2	38.1	88.2
Trueperella pyogenes	17.3	0	0	12.7	9.7
Pseudomonas aeruginosa	5.7	0	3.8	6.4	8.2
Clostridium spp.	2.2	0	0	3.9	8.6
Histophilus somni	2.8	0	1.62	3.1	12.7

NP – non-problems in the puerperium, GI – inflammations of genitalia

Nevertheless, up to 90% of recently calved cows develop a uterine infection in 1 to 2 weeks after calving. Most dairy cows have their first postpartum oestrous cycle in about 17 to 27 days after calving (Stevenson, 1997). These values were confirmed in both primipara and pluripara. Untreated primipara had this interval significantly longer (P <0.01; Table 2). This difference seems to have minimal effect on the term of the first insemination after parturition but will significantly affect days open period of the primipara and pluripara which were not preventively treated (P <0.01; Table 2).

The microflora occurring in the postpartum period is usually represented by aerobic as well as anaerobic species (*E. coli*, *Pseudomonas aeruginosa*, *Arcanobacterium pyogenes*, *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella multocida*, *Clostridium* sp. and *Histophilus somni*; Table 3), (Husain et al., 1990; Noakes et al., 1991; Bondurant, 1999; Zerbe, 2002; Williams et al., 2005; Sheldon et al., 2010). In the presented groups, the most prevalent pathogens were *E. coli* (74.7%) and *A. pyogenes* (17.3%). *E. coli* caused up to 88.2% of genital inflammation, mostly endometritis (Table 3). *E. coli* are commonly isolated in the early postpartum period (first 5 days postpartum) and are believed to favour infection of other opportunistic bacteria such as *F. necrophorum*, *A. pyogenes*, and *Bacteroides* spp. (Dohmen et al., 2000; Sheldon et al., 2002). Recent studies have characterized the uterine microbiota from cows (Machado et al., 2012) showing a different microbial community from metritic or healthy cows. They found that *Fusobacteria* was the dominant group in metritis cows, while *Gamaproteobacteria* was more present in the uterus of healthy cows. In addition to health risks, the postpartum inflammatory process also poses a risk of economic losses arising from lower production and the treatment costs. The intrauterine infusions of antibiotics can be controversial; however, some authors feel that they should be used for clinical endometritis depending on the severity of infections. Reduction of a load of potential bacterial pathogens in the uterus immediately after calving could reduce the risk of uterine disease (LeBlanc et al., 2011; Deng et al., 2015). For this reason, but also because of possible resistance, methods of antibiotics substitutions with other active preparations have been sought for a long time; they should be of ecological nature. The known protective effects of *Lactobacillus* spp. as natural complexes of the vaginal environment in humans (Bruce and Reid, 1988) have been used in this work as a preventive method to reduce the risk of bacterial puerperal infections. Table 3 shows the positive effect of the application of preparation based on *Lactobacillus* spp. to eliminate the occurrence of bacterial microflora. Preventive application of the preparation significantly eliminated the occurrence of bacterial microflora after the parturition to the 14th and 20th day of the puerperium, respectively. In Genís et al. (2018) study, the intra-vaginal application of lactic acid bacteria twice per week during the 3 week before calving was able to reduce the prevalence of metritis compared with no intervention cows too. The preventive application was manifested to a demonstrable extent also in the subsequent reproductive parameters of cows (Table 2). The preparation appeared to be more effective in primipara, especially concerning the incidence of inflammatory conditions of the genitals (P <0.01).

4 Conclusions

This work addresses the issue of prevention of the puerperal infections of cows with a preparation constructed based on the *Lactobacillus* spp. The lyophilised *Lactobacillus* spp. after dilution with a sterile solvent were applied 24 hours after parturition to 4,563 cows at 12 farms. Primiparous and pluriparous cows were used in the experiment. The treatment had a positive effect in reducing the incidence of postpartum microflora of the birth canal and in an evident improvement of the postpartum reproductive parameters. A more pronounced effect was observed in primiparous animals. The results show that the use of alternative drugs in the prevention of postpartum infections can reduce the number of antibiotics used in the treatment of postpartum problems in cows.

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