

Post Partum Bacteriology in Cows (Preliminary Date)



Healthcare

Keywords: inflammations, uterus, cows, uterine contaminants (Arcanobacterium pyogenes, Bacillus spp), puerperal metritis, lochia, Escherichia coli, etc.

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Abstract

The relationship of various clinical forms of uterine inflammation to bacterial contamination and the applicability of hydrogen peroxide for intrauterine treatment of clinical endometritis was the subject of this trial. Uterine contamination was compared among groups of cows according to clinical findings on days 6 ± 3 : MM, $n = 7$ (mild or severe puerperal metritis), SM, $n = 7$ (cows with severe puerperal metritis) and CM, $n = 7$ (cows without symptoms of puerperal metritis). After 21 ± 3 the seam cows (mild or severe clinical endometritis and controls without symptoms of the disease: MM, $n = 7$, SM, $n = 7$ and CM, $n = 7$). The applicability of 3% hydrogen peroxide was evaluated on cows after infusion 80 ml of the solution, clinical as well as bacteriological examination of uteri in cows suffering from postpartum pathology. A wider bacterial spectrum was found in the cows on day 6 ± 3 compared to day 21 ± 3 . Arcanobacterium pyogenes and Bacillus spp was the main uterine contaminant in cows suffering from all clinical types of uterine inflammation while this bacterium was not shown to be present in any of the control cows. The results show an important role of A. pyogenes in the etiopathogenesis of all clinical forms of uterine inflammations in postpartum cows and support the use of 3% hydrogen peroxide for intrauterine treatment of clinical endometritis even though sufficient antibacterial effects of the treatment are still to be confirmed.

1. Introduction

Inflammations of the uterus in cows, recently classified as puerperal metritis, clinical endometritis, subclinical endometritis, and pyometra represents one of the most important causes of (sub)infertility in dairy herds (Nakao et al., 1992; Huszenicza et al., 1999; LeBlanc et al., 2002a; Kim and Kang, 2003; Maizon et al., 2004; Gilbert et al., 2005; Sheldon et al., 2006) because the occurrence of various types of intrauterine puerperal metritis and clinical endometritis in herds usually reaches 20–40% and the occurrence of subclinical endometritis is probably even higher (Stevenson and Call, 1988; Peeler et al., 1994; Gilbert et al., 2005; Foldi et al., Veterinarni Medicina, 55, 2010 (10): 504–511 Original Paper 505 2006; Sheldon et al., 2006). Therefore intrauterine antimicrobial treatment represents a common and frequent procedure in dairy farms even though the results of the treatment are variable (Smith et al., 1998; Olson, 1996; Drillich et al., 2005; Dolezel et al., 2008).

Nevertheless, bacterial contamination of the uterus in early postpartum cows is common (Huszenicza et al., 1999) and the development of uterine inflammation depends on local immunity and on the intensity of contamination and the spectrum of contaminants (Foldi et al., 2006; Sheldon et al., 2009a, b). Thus an effective control of postpartum contamination of the uterus provides the chance to improve both fertility and general health condition of dairy herds. The purpose of this study was to compare uterine contamination in cows suffering from various clinical types of uterine inflammation with cows without any clinical symptoms of the disease and evaluation of the applicability of 3% hydrogen peroxide as a new agent for intrauterine treatment (Bekana M., et al (1994), Bekana M., et al (1996), Bellavite P. et al (1983), Biagi et al (1990).

2. Material and methods

Twenty one postpartum cows (Holstein and Jersey) from two commercial dairy farms with 200 and 250 housed cows were used in the experiment. Clinical examination was performed on 21 cows after parturition are diagnosing for puerperal metritis mostly during 2014-2015 years. The examination included manual vaginal examination with withdrawal and evaluation of secretion from the vagina and transrectal palpation of the uterus. All examined cows were divided into three experimental groups on the basis of postpartum period and clinical findings.

The groups were established as follows: CM (control cows without clinical symptoms of puerperal metritis – normal lochia, (n = 7), MM (cows with mild puerperal metritis – marked purulent lochia, n = 7), SM (cows with severe puerperal metritis – putrid lochia, n = 7). Uterine swabs for bacteriological examination are used. After inoculation the plates were incubated aerobically and anaerobically for 18 to 24 h at 37 °C and for a further 24 h if bacterial growth had not ensued. In group MM and SM is evaluation of the therapeutic effect of 3 % hydrogen peroxide 21 ± 3 days post partum.

3. Results and discussion

A wider bacterial spectrum and higher occurrence of *Escherichia coli* was found in the cows on day 6 ± 3 compared to day 21 ± 3. Namely, the occurrence of *E. coli* was higher at the earlier postpartum term, and the difference between groups SM and SE was significant ($P < 0.05$). The presence of *A. pyogenes* was not shown in any cow without clinical symptoms of uterine inflammation on day 6 ± 3 as well as 21 ± 3. In contrast, *A. pyogenes* was the most frequent contaminant of uteri in cows suffering from puerperal metritis as well as clinical endometritis; thus, the occurrence of this bacteria was significantly higher in Groups MM and SM compared to Group CM (4/7 and 5/7 vs. 0/7).

Table 1. Occurrence of uterine bacteria in the CM (cows without symptoms of puerperal metritis), MM (cows with mild puerperal metritis) and SM (cows with severe puerperal metritis) groups on day 6 ± 3 post partum CM (n = 7), MM (n = 7), SM (n = 7).

	CM (n = 7)	MM (n = 7)	SM (n = 7)
<i>A. pyogenis</i> (%)	0	56	84
<i>Bacillus spp</i> (%)	28	42	70
<i>E. Coli</i> (%)	14	28	28
<i>P. Mieabilis</i> (%)	14	14	42
<i>Staphilococcus CN</i> (%)	0	28	28

Table 2. Occurrence of uterine bacteria in the CM (cows without symptoms of clinical endometritis), MM (cows with mild clinical endometritis) and SM (cows with severe puerperal metritis) groups on Day 21 ± 3 post partum. CM (n = 7), MM (n = 7), SM (n = 7).

	CM (n = 7)	MM (n = 7)	SM (n = 7)
<i>A. pyogenis</i> (%)	0	14	14
<i>Bacillus spp</i> (%)	14	0	28
<i>E. Coli</i> (%)	0	0	0
<i>P. Mieabilis</i> (%)	0	0	0
<i>Staphilococcus CN</i> (%)	0	14	14

Table 3. Reproductive parameters in previously non-treated (CM) or treated (MM, SM) after intra uterine. administration of 3% hydrogen peroxide.

	CM (n = 7)	MM (n = 7)	SM (n = 7)
Calving to first service interval (days)	76 ± 16.2	94 ± 18.4	102 ± 8.0
First service pregnancy rate (%)	70	56	42
Services per conception (days)	1.7	2.2	2.6

Purulent or fetid secretions manually obtained from the vagina are usually considered to be the most important symptoms of postpartum uterine inflammation (Drillich et al., 2002; Zilaitis et al., 2004; Drillich, 2006). Accordingly, these symptoms were considered as key in the diagnosis of the uterine condition in our trial. We evaluated the fetid character of secretions as a more serious stage of inflammation compared to the purulent character on day 6 ± 3 post partum, and the purulent character of the secretion as a more serious stage compared to muco-purulent character on day 21 ± 3 post partum, because the content of pus in secretion reaching up to 50% and its gradual reduction in the course of the early post partum period is described as physiological (Dohmen et al., 1995; LeBlanc et al., 2002b; Williams et al., 2005). Thus, the diagnosis of a pathological condition of the uterus on the basis of the content of pus in a secretion obtained from the vagina before day 25 post partum is questionable. For this reason only abundant content ($> 50\%$) of pus was considered to be a symptom of mild puerperal metritis in the early postpartum period, and muco-purulent secretion (content of pus $< 50\%$) a symptom of mild clinical endometritis in a later period. Bacterial contamination of the postpartum uterus is common and decreases during puerperium and becomes contamination-free usually from day 40, even though in some cows without symptoms of uterine inflammation, bacterial contamination of the uterus can be observed until day 60 post partum (Youngquist RS, 1990; Schirar and Martinet, 1982). Although a similar course of uterine infection is described during physiological as well as pathological post partum involution, some differences were found in the quantity and spectrum of contaminants. (Endo) metritis in cows is usually associated with contaminants such as *E. coli*, *A. pyogenes*, *Fusobacterium necrophorum*, *Bacteroides melaninogenicus*, which show varied interactions (Foldi et al., 2006; Yavari et al., 2007; Azawi et al., 2008; Wang Jun et al., 2008; Petit et al., 2009). *E. coli* usually asserts itself at the beginning of inflammation and together with endotoxins (lipopolysaccharide) facilitates a subsequent infection with *A. pyogenes* (Dohmen et al., 2000; Zilaitis et al., 2004) and in addition inhibits follicular growth as well as the secretion of oestradiol (Williams et al., 2008a,b; Sheldon et al., 2009a). PMN phagocytosis has been shown to be inhibited in the presence of *E. coli* (Zerbe et al., 2001). In addition, a positive correlation between the occurrence of *A. pyogenes* and *Bacteroides* spp. or *F. necrophorum* has been described (Huszenicza et al., 1999). Occasionally, streptococci, staphylococci, *Proteus* or *Clostridium* spp. are also associated with (endo) metritis (Dohmen et al., 1995; Mateus et al., 2002).

Hydrogen peroxide represents a water soluble and mildly acidic fluid with strong oxidative properties and with the ability to inhibit many enzymatic processes (Musil, 1990). Above all it is used for disinfection and suppression of weak haemorrhage because of its antiseptic and haemostatic properties and easy permeation through organic membranes (Lullmann et al., 2004). In addition, foam created during the release of oxygen in the course of the hydrogen peroxide reaction affects the mechanical cleanup (Lullmann et al., 2004). Bactericidal, viricidal, and fungicidal effects of hydrogen peroxide have been described in detail (Mentel and Schmidt, 1973). Nevertheless, potentially detrimental effects of hydrogen peroxide on tissues and fibroblasts have also been reported (Mayes, 1998; Bagchi et al., 2007; Kim et al., 2009). Therefore, a maximum concentration of hydrogen peroxide 3% is recommended for internal administration due to its detrimental effects and risk of gass embole (Bagchi et al., 2007). The effect of the treatment under in vivo conditions was confirmed successively by the disappearance of clinical symptoms of uterine disorders in most treated cows, decrease of bacterial contamination and comparable reproductive parameters with controls. In conclusion our results show a wider spectrum of uterine bacteria in cows on day 6 ± 3 compared to day 21 ± 3 post partum, the domination of *A. pyogenes* in the uterus of cows suffering from mild as well as severe puerperal metritis or clinical endometritis and the applicability of 3% hydrogen peroxide for intrauterine treatment of clinical endometritis in cows even though a sufficient antibacterial effect of the treatment remains to be confirmed.

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