Bacterial contamination of the uterus in cows with various clinical types of metritis and endometritis and use of hydrogen peroxide for intrauterine treatment

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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 10 ± 3 (mild or severe puerperal metritis and controls without symptoms of the disease: MM, n = 16 or SM, n = 8 and CM, n = 13) and 25 ± 3 (mild or severe clinical endometritis and controls without symptoms of the disease: ME, n = 28 or SE, n = 40 and CE, n = 10). The applicability of 3% hydrogen peroxide was evaluated on the basis of macroscopic examination of intact and closed uteri from slaughtered cows after infusion of 50, 80, and 100 ml of the solution, clinical as well as bacteriological examination of uteri in cows suffering from clinical endometritis (Group E1 – treatment for the first time, n = 18 and Group E2 – previous treatment for retained placenta or puerperal metritis, n = 12) before and seven days after intrauterine administration of 80 ml of the solution as well as subsequent reproductive performance of treated cows in comparison with untreated controls without symptoms of the disease (Group C, n = 20). A wider bacterial spectrum was found in the cows on day 10 ± 3 compared to day 25 ± 3. *Arcanobacterium pyogenes* 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 (MM 7/16 and SM 6/8 vs. CM 0/13, $P < 0.05$ and $P < 0.01$; ME 14/28 and SE 18/40 vs. CE 0/10, $P < 0.05$). No macroscopic changes in uteri were found after infusion of various volumes of 3% hydrogen peroxide, only gas infiltration to the surrounding tissue occurred in completely closed uteri after deposition of 100 ml of the solution. Clinical symptoms of endometritis disappeared in 83% (E1) and 67% (E2) of affected cows and bacterial contamination decreased markedly (but not significantly) in both groups up to day 7 after intrauterine treatment. Reproductive parameters in treated cows compared to controls were not different. 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.

Keywords: postpartum cows; puerperal metritis; clinical endometritis; bacterial contamination; 3% hydrogen peroxide

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 (Sagartz and Hardenbrook, 1971; Markusfeld, 1987; Stevenson and Call, 1988; Peeler et al., 1994; Gilbert et al., 2005; Foldi et al.,

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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 (Gilbert, 1992; Whitacre, 1992; Montes and Pugh, 1993; 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 (Experiment I) and evaluation of the applicability of 3% hydrogen peroxide as a new agent for intrauterine treatment of clinical endometritis (Experiment II).

MATERIAL AND METHODS

Experiment I

One hundred and fifteen postpartum cows (Holstein) from two commercial dairy farms with 500 and 800 housed cows were used in the experiment. Clinical examination was performed on 37 cows on day 10 ± 3 with the aim of diagnosing puerperal metritis and in 78 cows on day 25 ± 3 post partum with the aim of diagnosing clinical endometritis mostly during July and August. 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 six 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 = 13), MM (cows with mild puerperal metritis – marked purulent lochia, n = 16), SM (cows with severe puerperal metritis – putrid lochia, n = 8), CE (control cows without symptoms of clinical endometritis – clean mucus, complete involution of uterus, n = 10), ME (cows with mild clinical endometritis – mucopurulent secretion, complete or almost complete involution of uterus, n = 28), and SE (cows with severe clinical endometritis – purulent secretion, incomplete involution of uterus, n = 40).

Uterine swabs for bacteriological examination (Uterine Culture Swab, EQUI-VET) were aseptically collected from each cow, the samples were immediately inserted into the transport media Amies (CM425; Oxoid, Basingstoke, UK) and were transported to the university laboratory within 3 h after collection, where they underwent bacteriological examination. Individual swabs were cultured on Columbia agar (CM331; Oxoid, Basingstoke, UK) containing 5% citrated sheep blood and MacConkey agar (CM115; Oxoid, Basingstoke, UK). 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. Bacteriological routine diagnostic procedures including Gram-staining, catalase-testing and biochemical confirmation by diagnostic kits (Micro-La Test, Pliva-Lachema Diagnostika, Brno, Czech Republic) were used for culture identification.

Differences in bacterial contamination among the groups were evaluated using the Chi-square test.

Experiment II

Part 1 – Estimation of an adequate dose of 3% hydrogen peroxide for *in vitro* conditions. Twelve involuted uteri from slaughtered dairy cows were used in the test. Uterotubal junctions were ligated in six uteri. Fifty, 80 and 100 ml of 3% hydrogen peroxide were infused into the uteri transcervically using a catheter (each volume was infused into two intact and into two ligated uteri). Internal uterine orifices were closed by forceps immediately after the infusion in six ligated uteri. Macroscopic examination of uteri was performed 0, 15, 30, 60 and 180 min after infusion of the solution. The uteri were opened and the endometrium was macroscopically examined at the end of observation. Eighty milliliters were determined to be an adequate volume of the solution for intrauterine administration (see Results).

Part 2 – Evaluation of the therapeutic effect of 3% hydrogen peroxide under *in vivo* conditions. Thirty dairy cows (Holstein) 22–28 days post partum suffering from clinical endometritis diagnosed by rectal and vaginal examination (see Experiment I) were used in the trial. Involution of the uterus and quality of the secretion manually withdrawn from

the vagina were evaluated and a marked content of pus in the secretion was considered to be the main marker of endometritis. The levels of clinical endometritis were not assessed. Intrauterine administration of 80 ml of 3% hydrogen peroxide was performed in these cows immediately after clinical examination. The cows were either treated for the first time (Group E1, n = 18) or had been treated previously for retained placenta or puerperal metritis (Group E2, n = 12). A control group (C, n = 20) consisted of non-treated cows without symptoms of the disease in the same postpartum period. Bacteriological (eight cows in groups E1 and E2 and four cows in group C) examination of uteri (see Experiment I) were performed before treatment and seven days later after the 2nd clinical examination. In addition, calving to first service interval, first service pregnancy rate, calving to conception interval, services per conception, and pregnancy by day 100 and 150 post partum were compared among the groups.

Statistical evaluation of the differences in clinical and bacteriological findings was performed using Fisher's exact test, and reproductive parameters were compared using the Cruscal-Wallis test.

RESULTS

Experiment I

A wider bacterial spectrum and higher occurrence of *Escherichia coli* was found in the cows on day 10 ± 3 compared to day 25 ± 3. Namely, the occurrence of *E. coli* was higher at the earlier postpartum term, and the difference between groups SM and SE was significant (2/8 vs. 0/40, $P < 0.05$). The presence of *A. pyogenes* was not shown in any cow without clinical symptoms of uterine inflammation on day 10 ± 3 as well as 25 ± 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 (7/16 and 6/8 vs. 0/13) and similarly in Groups ME and SE compared to Group CE (14/28 and 18/40 vs. 0/10) (Tables 1 and 2).

Experiment II

Part 1. Greater distension was found in closed uteri compared to intact uteri after deposition of 50, 80, and 100 ml of 3% hydrogen peroxide. Plentiful outlet of the solution with gas from the cervix of intact (open) uteri occurred after intrauterine deposition (Figure 1). Nevertheless, no macroscopic injuries were found in any examined uteri but symptoms of gas infiltration to the surrounding tissue were observed in closed (ligated) uteri after deposition of 100 ml of the 3% hydrogen peroxide (Figure 2). On the basis of these results 80 ml of the solution was determined as the maximum volume applicable for intrauterine treatment in cows.

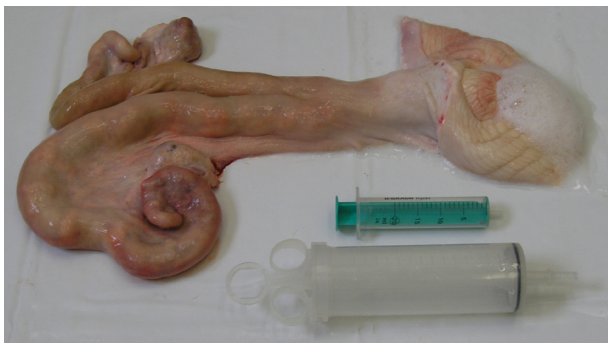

Figure 1. Discharge after intrauterine deposition of 100 ml of 3% hydrogen peroxide

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 10 ± 3 post partum

	CM (n = 13)	MM (n = 16)	SM (n = 8)
A. pyogenes (%)	0[ab]	44[a]	75[b]
Bacillus spp. (%)	46	13	25
E. coli (%)	23	0	25
P. mirabilis (%)	15	6	13
Staphyloccocus CN (%)	0	13	13

[a]$P < 0.05$; [b]$P < 0.01$

Table 2. Occurrence of uterine bacteria in the CE (cows without symptoms of clinical endometritis), ME (cows with mild clinical endometritis) and SE (cows with severe clinical endometritis) groups on Day 25 ± 3 post partum

	CE (n = 10)	ME (n = 28)	SE (n = 40)
A. pyogenes (%)	0[ab]	50[a]	45[b]
Bacillus spp. (%)	20	7	8
E. coli (%)	0	0	0
P. mirabillis (%)	0	0	0
Staphyloccocus CN (%)	10	7	5

[a]$P < 0.05$; [b]$P < 0.05$

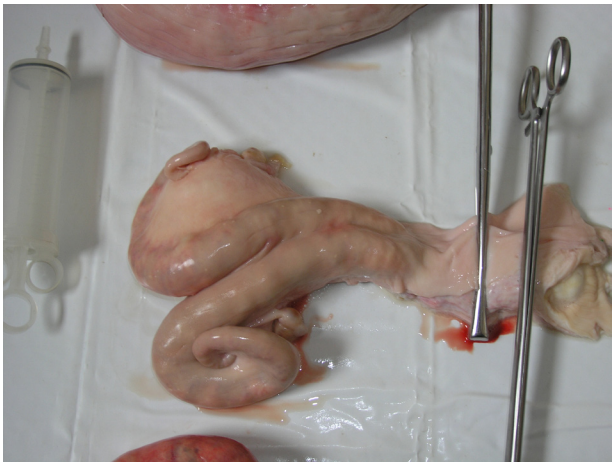

Figure 2. Infiltration of gas to the surrounding tissue after intrauterine deposition of 100 ml of 3% hydrogen peroxide

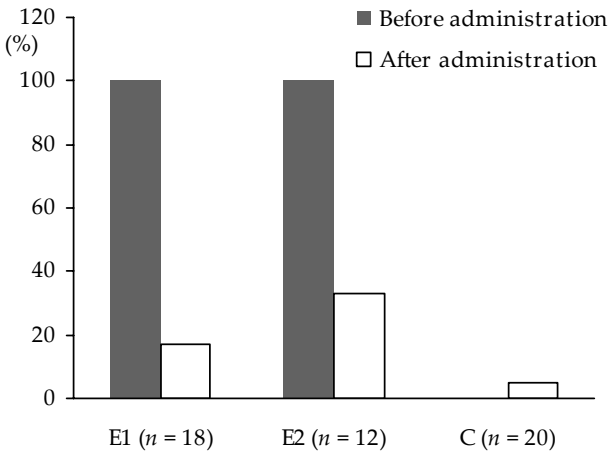

Figure 3. Occurrence (%) of clinical symptoms in previously non-treated (E1) or treated (E2) cows suffering from clinical endometritis on day 7 after *i.u.* administration of 3% hydrogen peroxide and in controls (C)

Part 2. Clinical symptoms of endometritis disappeared in 15 out of 18 (83%, $P < 0.05$) and eight out of 12 (67%, $P < 0.05$) of the cows in Groups E1 and E2, respectively, on day 7 after treatment (Figure 3). These symptoms also occurred in 1 untreated control cow from control Group C.

Similarly, total infection (Figure 4) and infection with *A. pyogenes* (Figure 5) in treated cows decreased approximately by about 50% but differences were not significant. Surprisingly, negative bacteriological findings were found in all cows without clinical symptoms of endometritis at both examinations.

Table 3. Reproductive parameters in previously non-treated (E1) or treated (E2) cows suffering from clinical endometritis after *i.u.* administration of 3% hydrogen peroxide and in controls (C)

	E1 (n = 18)	E2 (n = 12)	C (n = 20)
Calving to first service interval (days)	83 ± 25.4	92 ± 25.3	73 ± 14.0
First service pregnancy rate (%)	39	25	50
Calving to conception interval (days)	103 ± 32.6	125 ± 38.5	106 ± 48.2
Services per conception (days)	1.6 ± 0.50	2.2 ± 1.11	2.0 ± 1.41
Pregnancy until day 100 (%)	50	25	55
Pregnancy until day 150 (%)	94	83	85

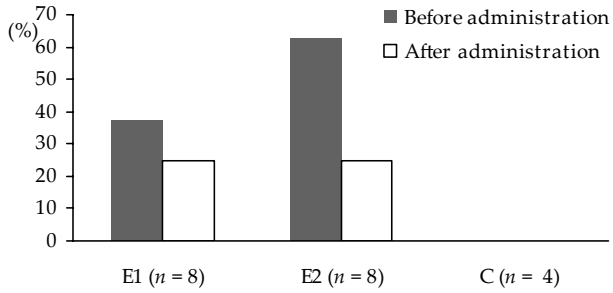

Figure 4. Occurrence (%) of uterine contamination in previously non-treated (E1) or treated (E2) cows suffering from clinical endometritis before and on day 7 after *i.u.* administration of 3% hydrogen peroxide and in controls (C)

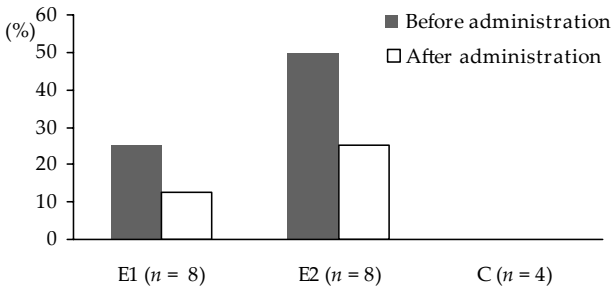

Figure 5. Occurrence (%) of *A. pyogenes* in the uteri of previously non-treated (E1) or treated (E2) cows suffering from clinical endometritis before and on day 7 after *i.u.* administration of 3% hydrogen peroxide and in controls (C)

Even though all reproductive parameters were generally worse in Group E2 in comparison with Groups E1 and C, the differences were not significant (Table 3).

DISCUSSION

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 10 ± 3 post partum, and the purulent character of the secretion as a more serious stage compared to muco-purulent character on day 25 ± 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 (Kudlac and Vlcek, 1970; Studer and Morrow, 1981; Schirar and Martinet, 1982; Lofstedt, 1984; Hussain et al., 1990; Zerbe et al., 1996). 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* (Watson, 1989; Zerbe et al., 2001). In addition, a positive correlation between the occurrence of *A. pyogenes* and *Bacteroides* spp. or *F. necrophorum* has been described (Bekana et al., 1994; Dohmen et al., 1995; 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). Accordingly, with these data we observed *E. coli* only on day 10 ± 3 but did not find it in any cow on day 25 ± 3 post partum while *Bacillus* spp. were found in all experimental groups regardless of the term

post partum. Convincing findings supporting the results of the above mentioned reports were made for *A. pyogenes*. We isolated this bacterium only in cows suffering from (endo)metritis. The occurrence of *A. pyogenes* ranged from 44 to 75% in the individual groups of affected cows. Thus, we unambiguously show *A. pyogenes* to be the main uterine contaminant in cows suffering from (endo)metritis. Thus, the presence of this bacterium can be considered as an indicator of a pathological condition in the bovine uterus. Therefore, in our trial, a high occurrence of this bacterium was associated with inflammation also in cows with purulent secretion on day 10 ± 3 as well as in cows with mucopurulent secretion on day 25 ± 3 post partum. Associations of fetid secretion with *A. pyogenes*, *E. coli*, non-hemolytic streptococci and *Mannheimia haemolytica* and mucopurulent or purulent secretion with *A. pyogenes*, *Proteus* and *F. necrophorum* have been described previously (Williams et al., 2005).

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 (Youngquist, 1990; 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 (Wenke et al., 1977; 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; Yu et al., 2008; Kim et al., 2009; Silva 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 (Wenke et al., 1977; Bagchi et al., 2007). This concentration was used in our trial but our results cannot be compared with other authors because we have not found any data regarding intrauterine administration of this solution. Firstly, an adequate volume had to be determined because oxygen released during the reaction could extremely distend the uterus and mechanically damage the uterine wall. Macroscopic examination of isolated uteri after infusion of the solution was considered to be a sufficiently accurate method for this purpose in our trial. 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 10 ± 3 compared to day 25 ± 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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