Pharmacokinetics of ceftiofur in plasma and uterine secretions and tissues after subcutaneous postpartum administration in lactating dairy cows

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Okker, H., Schmitt, E. J., Vos, P. L. A.M., Scherpenisse, P., Bergwerff, A. A., Jonker, F. H. Pharmacokinetics of ceftiofur in plasma and uterine secretions and tissues after subcutaneous postpartum administration in lactating dairy cows. *J. vet. Pharmacol. Therap.* **25,** 33–38.

A study was conducted to measure concentrations of potentially active ceftiofur derivatives, in plasma, in uterine tissues (endometrium and caruncles) and in uterine secretions at different time points after a single subcutaneous administration of ceftiofur hydrochloride (Excenel® RTU Sterile Suspension) at the dose of 1 mg/kg body weight in Holstein–Friesian dairy cows. The animals ($n = 4$) were injected within 24 h of calving, after expulsion of the foetal membranes. Plasma, lochial fluid, caruncles and endometrium were collected before ceftiofur hydrochloride administration and at 1, 2, 4, 8, 12 and 24 h after treatment. For each cow the concentrations of ceftiofur in the biological matrices were quantified using an high-performance liquid chromatography (HPLC) assay. The limit of quantification of the method was 0.1 μg/mL for plasma and 0.1 μg/g for lochial fluid, caruncles and endometrium. The concentrations of potentially active ceftiofur derivatives detected in plasma reached a maximum of 2.85 ± 1.11 μg/mL at 2 h and decreased to 0.64 ± 0.14 μg/mL at 24 h after administration. In lochial fluid, these concentrations reached a maximum of 0.97 ± 0.25 μg/g at 4 h and decreased to 0.22 ± 0.21 μg/g at 24 h after administration. In endometrium, these concentrations reached a maximum of 2.23 ± 0.82 μg/g at 4 h and decreased to 0.56 ± 0.14 μg/g at 24 h following the injection, whereas these levels in caruncles were 0.96 ± 0.45 and 0.60 ± 0.39 μg/g obtained at 8 and 24 h, respectively. At the dose of 1 mg/kg body weight in healthy dairy cows, subcutaneous administration of ceftiofur (as ceftiofur hydrochloride) after parturition results in concentrations of ceftiofur derivatives in uterine tissues and in lochial fluid that exceed the reported minimal inhibitory concentrations (MICs) for the common pathogens (*Escherichia coli*, *Fusobacterium necrophorum*, Bacteroides spp., and *Arcanobacterium pyogenes*) associated with acute puerperal metritis.

(Paper received 20 November 2000; accepted for publication 24 August 2001)

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INTRODUCTION

It is generally accepted that during the process of uterine involution in healthy cattle, bacterial contamination can last for up to 6 weeks after parturition (Elliot & McMahon, 1968; Griffin *et al.*, 1974; Paisley *et al.*, 1986; Bekana *et al.*, 1994; Dohmen *et al.*, 1996). In the early postpartum period, overgrowth of this bacterial population may result in a serious uterine infection, and eventually lead to a septicaemia or a severe toxic condition. This potentially life-threatening condition, called acute puerperal metritis, occurs during the first 14 days after parturition. Metritis is defined as the inflammation of the muscular and endometrial layers of the uterus. Affected cows may have an elevated body temperature and a fetid watery uterine discharge. Cows become anorexic and decreased milk production and economic losses have been reported (Deluyker *et al.*, 1991; van Verwen *et al.*, 1992; Rajala & Gröhn, 1998; Gröhn & Rajala-Schultz, 2000). Acute puerperal metritis is one of the most difficult reproductive tract infections to treat and there is agreement that systemic administration of an antimicrobial is needed (Sandals *et al.*, 1979; Bartlett *et al.*, 1986; Bretzlaff, 1987; Stevenson & Call, 1988).

Eschericha coli, *Arcanobacterium pyogenes* and Gram-negative anaerobes can be isolated routinely from the uterus of cows diagnosed with acute puerperal metritis before day 14 after parturition (Lohuis, 1998). Coliform flora and especially *E. coli* are present in 91% of the samples collected from affected animals in the week following parturition (Lohuis, 1998) and this frequency decreases for cases of metritis evaluated later after parturition. More severely affected cows have growth of obligatory anaerobic bacteria in the uterus, and a possible synergetic action of *A. pyogenes*, Bacteroides spp. and *Fusobacterium necrophorum* has been suggested (Olson *et al.*, 1984; Paisley *et al.*, 1986; Dohmen *et al.*, 1995).

As the uterine pathogenic flora changes with time, the efficacy of any given antibiotic treatment changes with the time of the treatment after parturition and with the severity of the disease. The chosen antibiotic should be efficacious in an anaerobic environment and in the presence of organic debris. In general, intrauterine administration of tetracycline has been used for the treatment of uterine infections (Bretzlaff, 1987). However, a significant incidence of resistance of uterine pathogens to oxytetracycline has been reported (Lohuis, 1998) and its efficacy in the treatment of acute puerperal metritis is therefore likely to be reduced.

Additionally, intrauterine antibiotic treatment does not allow therapeutic concentration of antimicrobials in other parts of the genital tract such as ovaries, oviducts and uterine wall (Bretzlaff, 1987). Oviducts do not resolve inflammation rapidly and antibiotics supportive of the immune reaction may be essential to combat oviductory infections. For instance, in an abattoir study prevalence of salpingitis was 16.3% in cows (Gonzales *et al.*, 1985). Parenteral antibiotic treatment may provide adequate therapeutic concentrations to all portions of the female genital tract. Acute puerperal metritis is associated with general illness and septicaemia and so parenteral antibiotic treatment is indicated.

Cephalosporins have been recommended for the parenteral and local treatment of uterine infections in cattle (Chastant-Maillard & Aguer, 1998). Ceftiofur is a third generation cephalosporin with bactericidal activity. The third generation cephalosporins, typically containing an aminothiazole group, are active against Gram-negative bacteria, retain good activity for Gram-positive bacteria and are known to be resistant to β-lactamase enzymes (Prescott & Baggot, 1993). Relatively high concentrations of ceftiofur have been found *in utero* after parental treatment of mares (Jonker, 1997).

The metabolism of ceftiofur is complex. Upon injection, ceftiofur is rapidly metabolized to desfuroylceftiofur which contains a free sulphydryl moiety, and furoic acid (Jaglan *et al.*, 1989; Beconi-Barker *et al.*, 1995). Desfuroylceftiofur contains an intact β-lactam ring and is the main microbiologically active residue of ceftiofur. Desfuroylceftiofur is microbiologically equipotent to ceftiofur against most veterinary pathogens (Salmon *et al.*, 1996). Binding of ceftiofur to cysteine or glutathione residues or to form dimers of desfuroylceftiofur is reversible (Jaglan *et al.*, 1989).

The objective of this study was to characterize the concentrations and decline of ceftiofur derivatives in plasma, uterine tissues (endometrium and caruncles) and uterine secretions of healthy Holstein–Friesian dairy cows following parturition after a single subcutaneous administration of ceftiofur hydrochloride at the dose of 1 mg/kg body weight.

MATERIALS AND METHODS

Experimental design

This study was approved by a local ethics committee according to the Legal Act on Animal Experiments of the Dutch Law and was conducted at the Department of Farm Animal Health at the Faculty of Veterinary Medicine in Utrecht, the Netherlands.

Six multiparous adult Holstein–Friesian dairy cows in their ninth month of gestation purchased at different Dutch dairy farms entered in the study. A minimum of a 14-day acclimatization period was allowed before calving and entrance in the study. Cows were identified with the letters A to F according to the order of calving. Cows weighed between 560 and 730 kg and calved spontaneously between 18 February and 23 March 2000.

Two cows, A and F, had retained foetal membranes, i.e. placenta was not expelled within 6 h after parturition (van Verwen *et al.*, 1992) and were removed from the study. During the 14 days before calving and until the end of the study period, none of the cows included in the study received antimicrobial, anti-inflammatory, oestrogenic, prostaglandin or any other pharmaceutical treatment other than with the test article.

The time of expulsion of the placenta for the four remaining cows of the test group took place at about 4 h after parturition.

Test material. Ceftiofur was administered in the form of the commercially available preparation of ceftiofur hydrochloride in a sterile oil suspension (Excenel® RTU Sterile Suspension; lot number 99G1624DDT, expiration date 07/2001, Pharmacia & Upjohn, Puurs, Belgium) containing 50 mg ceftiofur equivalents/mL at the dose of 1 mg/kg body weight or 1 mL for 50 kg body weight.

Treatment. To allow for an easier sample collection through an open cervix and to minimize animal discomfort, the study started immediately after expulsion of the placenta.

After parturition and directly after expulsion of the placenta, the cows were weighed on a calibrated scale. Upon weighing, cows were restrained and received a subcutaneous administration of the test article in the neck region at a dose of 1 mg/kg body weight. No more than 10 mL of product was administered per injection point. A new vial was opened for each cow entering the study.

Sampling. For each cow, sampling was performed in the following sequence: (1) blood, (2) lochial fluid, (3) caruncle and finally (4) endometrium. Lochial fluid was sampled before removing the caruncle and endometrium biopsy to avoid contamination with blood from the biopsy procedure. For blood, lochial fluid, caruncles and endometrium baseline samples were collected immediately before ceftiofur hydrochloride

administration (time point 0). Additional samples were collected 1, 2, 4, 8, 12 and 24 h after treatment with ceftiofur hydrochloride. The frequency of uterine tissue sampling did not result in excessive uterine bleeding or irritation during the course of the study. Blood was collected from the jugular vein in sterile heparinized Vacutainer® tubes (Becton & Dickinson, Le Pont de Claix, France). After collection, blood samples (2 × 10 mL tubes) were immediately centrifuged at 3500 ***g*** for 10 min at room temperature to obtain plasma. Aliquots of 1.5–2 mL plasma were stored in screw-capped tubes at –20 °C until analysis.

Lochial fluid samples of approximately 30 mL each were collected in a 50-mL Sarstedt tube. Tubes were introduced by hand into the uterus and lochial fluid was sampled from the pregnant horn. To facilitate the operator's work, a sterile lubricating gel was applied on the operating hand and arm. Lochial fluid was aliquoted in two parts of approximately 15 mL each and stored at –20 °C within 1 h after sampling.

Biopsies were taken with a Yeomen biopsy apparatus. The biopsy apparatus was heat-sterilized between use in different cows.

Caruncle samples were collected from the pregnant horn with the Yeomen biopsy apparatus, which was introduced and guided by hand into the uterus. When a large caruncle was localized (approximately 8 × 8 cm in diameter), a biopsy was taken from the caruncle surface. After thoroughly cleaning the biopsy-apparatus in chlorhexidine solution, the endometrium of the pregnant horn was sampled with the same biopsy-apparatus.

For each cow a different caruncle and endometrial fold was sampled at each time point. Endometrium and caruncle samples were blotted dry and placed into a 15-mL screw-capped tube; all uterine tissue samples were stored at –20 °C within 1 h after sampling.

Analytical methods. An established, validated high-performance liquid chromatography (HPLC) method was used for the determination of ceftiofur and desfuroylceftiofur related metabolites (called 'ceftiofur derivatives') (Beconi-Barker *et al.*, 1995). This method was modified and validated for the detection of ceftiofur derivatives in lochial fluid and endometrium.

In short, 1.0 mL plasma was mixed with 5 mL 50 mM potassium tetraborate at pH 9.0 containing 0.5 M sodium chloride and 130 mM dithioerythritol, and incubated at 50 °C for 15 min with intermittent mixing at every 5 min interval. Following reduction, 5 mL 0.1 M ammonium acetate containing 0.2 M iodoacetamide was added, mixed and incubation was continued at ambient temperature for 30 min in the dark under gentle agitation at 500 r.p.m. An aliquot of 1.0 g lochial fluid or caruncle, or 0.5 g endometrium was mixed with 5 mL 50 mM potassium tetraborate at pH 9.0 containing 0.5 M sodium chloride and 25 mM dithioerythritol. After homogenization using a Potter–Elvehjem (lochial fluid) or microultraturrax (caruncle and endometrium), incubation was carried out as described for plasma. The reaction was quenched by addition of 3 mL 0.76 M iodoacetamide in 25 mM potassium dihydrogenophosphate adjusted to pH 7.0 with sodium hydroxide for 30 min under identical conditions as for plasma. Suspensions were acidified by addition of 375 µL fivefold diluted phosphoric acid. After centrifugation at 3500 ***g*** for 25 min or at 65 000 ***g*** for 20 min in the case of lochial fluid, at 5 °C, supernatants were transferred onto preconditioned C_{18} solid-phase extraction (SPE) cartridges (1 g; Bond Elut, Varian). Cartridges were washed with 5 mL 0.1 M ammonium acetate and 5 mL 2% (v/v) acetic acid, and then eluted with a mixture of acetonitrile and 2% (v/v) acetic acid at 2:8 (v/v). Analyte-containing eluates were passed through activated and dried SCX-SPE cartridges (100 mg; Bond Elut, Varian). The SCX-retained DCA was eluted with 500 µL (plasma), 750 µL (endometrium and caruncle) or 1.00 mL (lochial fluid) of a mixture of 1.0 M ammonium acetate and acetonitrile at 85:15 (v/v).

High-performance liquid chromatography analysis of 100 µL samples was carried out on a combination of 3 µm C_{18} (50 × 4.6 mm; Phenomenex) and 3 µm phenyl-hexyl (50 × 4.6 mm; Phenomenex) columns connected in line. Elution of analytes was performed using a binary linear gradient using 10 mM ammonium acetate at pH 6.8 (eluent A) and acetonitrile (eluent B) at a flow rate of 1.0 mL/min as follows: 99% A (v/v) for 1.9 min, to 92% A (v/v) in 0.1 min, to 82% A (v/v) in 12 min. The HPLC-eluate was monitored at 266 nm.

All samples were analysed for the presence of ceftiofur derivatives. The limit of quantification of the method was 0.1 µg/g (endometrium, caruncles and lochial fluid). For plasma the lower limit was 0.1 µg/mL.

Data analysis

For each animal and each of plasma, lochial fluid, caruncle and endometrial tissues the area under the ceftiofur concentration–time curve (*AUC*), the maximum concentration (C_{max}) and the time at which the maximum concentration occurred (T_{max}) were determined. The *AUC*s were calculated by summing the areas of the trapezoids formed by adjacent concentration–time points. The C_{max} was determined as the largest value among the ceftiofur concentrations and T_{max} was set to the time at which the largest value occurred. For each of plasma, lochial fluid, caruncle and endometrial tissues the mean and standard deviations were calculated.

RESULTS

Analytical results are reported in Tables 1 and 2 and Fig. 1.

Table 1. Kinetic parameters of ceftiofur detected as desfuroylceftiofur-acetamide in plasma of dairy cows after subcutaneous administration of ceftiofur hydrochloride at the dose of 1 mg/kg body weight

Parameters	Mean ± SD	Range
AUC (h µg/mL)	36.52 ± 8.19	29.41–46.97
C_{max} (µg/mL)	2.85 ± 1.11	1.61–4.26
T_{max} (h)	2 ± 0	2–2

AUC = area under the curve, C_{max} = peak concentration, T_{max} = time to peak concentration.

Table 2. Kinetic parameters of ceftiofur detected as desfuroylceftiofur-acetamide in lochial and uterine tissues of dairy cows after subcutaneous administration of ceftiofur hydrochloride at the dose of 1 mg/kg body weight

	Lochial fluid		Caruncle		Endometrium	
Parameters	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
AUC (h μg/g)	13.73 ± 3.67	10.85–19.11	17.66 ± 5.27	13.56–25.09	28.79 ± 6.11	23.43–35.67
C_{max} (μg/g)	0.98 ± 0.25	0.82–1.34	1.11 ± 0.24	0.88–1.45	2.25 ± 0.79	1.44–3.09
T_{max} (h)	5 ± 2	4–8	11.5 ± 9.3	2–24	5 ± 2	4–8

AUC = area under the curve, C_{max} = peak concentration, T_{max} = time to peak concentration.

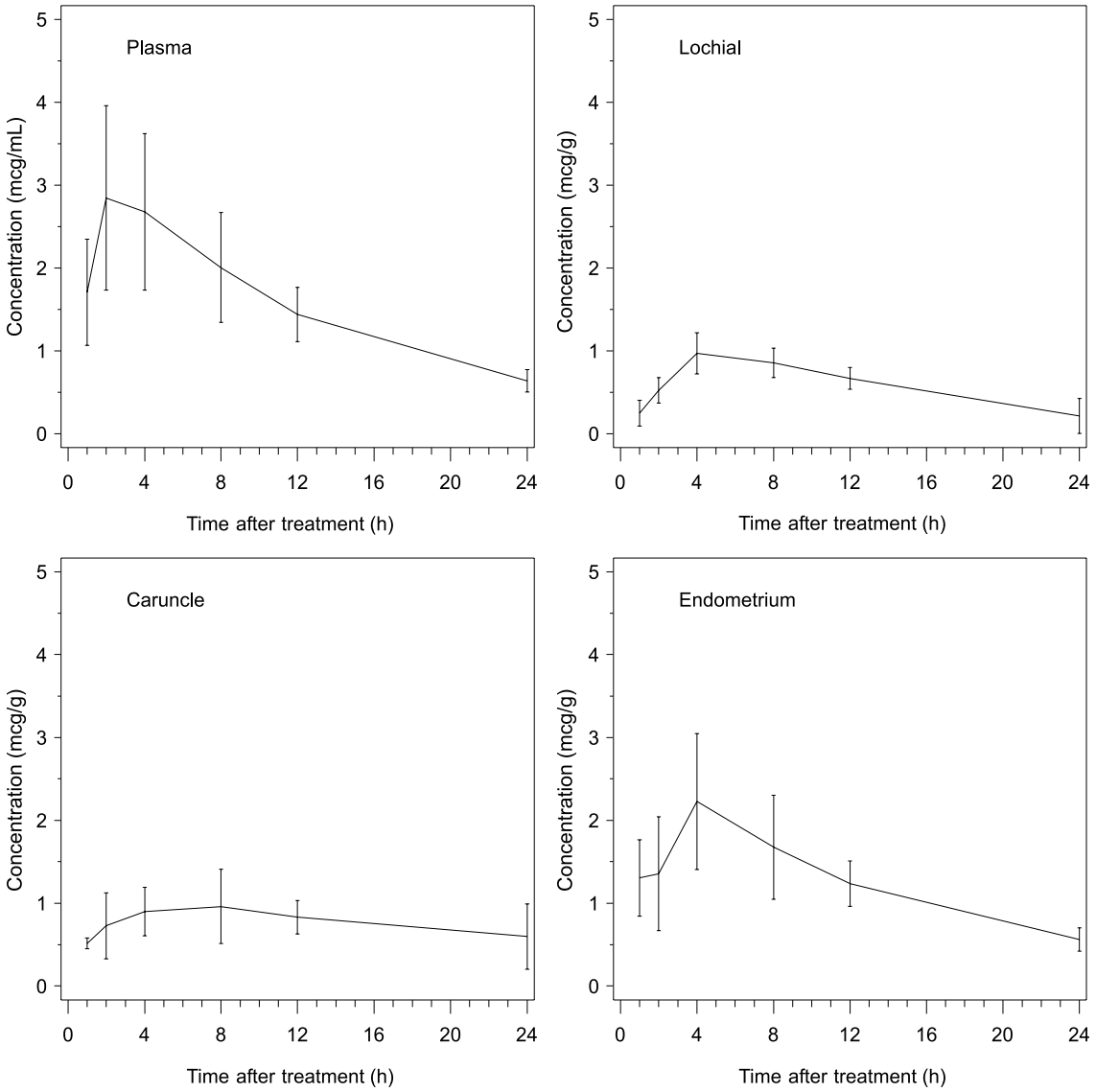

Fig. 1. Mean concentration of ceftiofur detected as desfuroylceftiofur-acetamide and active metabolites in plasma (μg/mL), lochial fluid (μg/g), uterine tissues [endometrium (μg/g) and caruncle (μg/g)] after subcutaneous administration of ceftiofur hydrochloride at the dose of 1 mg/kg in dairy cows [mean ± SD ($n = 4$ cows)].

Plasma. The concentration of ceftiofur derivatives in plasma reached a maximum of 2.85 ± 1.11 μg/mL 2 h after injection and decreased during the following 22 h to reach 0.64 ± 0.14 μg/mL at 24 h after treatment (Fig. 1).

Lochial. Mean concentrations of ceftiofur derivatives in lochial fluid of the four cows, increased to a peak concentration of 0.97 ± 0.25 μg/g at 4 h and decreased to 0.22 ± 0.21 μg/g at 24 h after treatment (Fig. 1).

Mean concentrations of ceftiofur in caruncle tissue reached a maximum of 0.96 ± 0.45 μg/g at 8 h and decreased to 0.60 ± 0.39 μg/g 24 h after administration (Fig. 1).

The decline of ceftiofur derivatives concentrations in endometrium showed a pattern similar to what was observed with plasma. The highest level in endometrium was found at 4 h after treatment. Mean concentrations for the cows in the study varied between 2.23 ± 0.82 μg/g at 4 h and 0.56 ± 0.14 μg/g at 24 h after treatment (Fig. 1).

DISCUSSION

The peak concentration in plasma observed in the present study (2.85 ± 1.11 μg/mL) is 62% of the maximum concentration (4.58 μg/mL) reported by Soback *et al.* (1989) after intramuscular administration of 2 mg/kg of ceftiofur sodium to dairy cows.

At present, there are no other published reports on uterine concentrations of ceftiofur in the cow. In mares, Cervantes *et al.* (1993), using a microbiological method, did not detect antimicrobial activity in endometrium after repeated administration of ceftiofur sodium at the dose of 2 mg/kg body weight. In contrast, Jonker (1997) detected a high level of ceftiofur in mares' endometrium (1.23 μg/mL of ceftiofur derivatives at 1 h after treatment) by HPLC analysis after a single intramuscular administration of ceftiofur sodium at the dose of 2 mg/kg body weight. The results reported in the present study support the earlier results of Jonker (1997) in the mare.

In the present study, a lower concentration of ceftiofur was detected in caruncle and lochial fluid when compared with the concentrations detected in plasma and endometrium.

The surface of the caruncle undergoes ischaemic necrosis immediately after parturition (Bondurant, 1999). Reduced blood perfusion of the caruncles might explain lower concentrations. The lower concentrations of ceftiofur in lochial fluid compared with plasma and endometrial tissue may be because of dilution from fluids remaining in the uterine cavity after parturition. In view of this, it is remarkable that concentrations in excess of 0.3 μg/g were observed at every tested time point in lochial fluid. The levels of ceftiofur found in the lochial fluid are likely to be effective against bacteria in the uterine lumen. This may have an additional effect on the outcome of the antibiotic therapy: the primary objective of parenteral antibiotic therapy in cases of acute metritis is to control the deleterious effects of septicaemia and/or severe toxic condition caused by *E. coli* and to prevent systemic invasion from bacteria of uterine origin (Bretzlaff, 1987).

The bacteria in acute postpartum metritis are not intracellular organisms. Therefore, plasma concentrations of ceftiofur, which are similar to extracellular fluid concentrations are the best predictor of potential effects of the drug on the target pathogens disrupting the endometrium.

Management of polymicrobial infections caused by aerobic and anaerobic bacteria is complex because of the large spectrum of bacteria involved in the infectious process and their various antibiotic susceptibility pattern (Brook, 1995). An early treatment of acute puerperal metritis is essential to control the deleterious effects of septicaemia and/or severe toxic conditions caused by *E. coli* (elevated body temperature, reduced milk production, reduced feed intake) and to limit proliferation of microaerophilic (*A. pyogenes*) and anaerobic (*F. necrophorum* and Bacteroides spp.) bacteria. Treatment should allow the animal's immune system to overcome the infection. This is consistent with the observation that the uterus will remain contaminated with bacteria for at least 2–3 weeks after the end of treatment (Dohmen *et al.*, 1996).

The minimal inhibitory concentration of ceftiofur for *E. coli*, *F. necrophorum*, Bacteroides spp. and *A. pyogenes* are reported in Table 3.

The ceftiofur concentrations reported in the present study were measured in healthy cows immediately after parturition. It can be hypothesized that the concentration of ceftiofur is higher at the uterine level in cows with acute puerperal metritis than in healthy cows. Clarke *et al.* (1996), who used microbiological and HPLC analytical assays, reported that the ceftiofur concentrations were higher at bacterially induced inflammatory sites. This was probably because of the accumulation of inflammatory proteins at the infection site. These accumulating acute-phase inflammatory proteins may reversibly bind ceftiofur and increase the local concentration of this antibiotic. It is therefore likely that a similar phenomenon may increase the ceftiofur concentrations in the uterus during the inflammatory process characteristic of acute puerperal metritis.

Table 3. Minimal inhibitory concentrations (*MIC*) of ceftiofur for *Escherichia coli*, *Arcanobacterium pyogenes* and *Fusobacterium necrophorum*

	MIC (μg/mL)				
Bacteria identification	No. of isolates	Range	MIC_{50}	MIC_{90}	Bibliographical references
Fusobacterium necrophorum	17	≤ 0.06	≤ 0.06	≤ 0.06	Samitz *et al.* (1996)
Bacteroides fragilis group	29	≤ 0.06–≥ 16	1	16	Samitz *et al.* (1996)
Escherichia coli	42	n.a.	n.a.	≤ 0.5	Cervantes *et al.* (1993)
E. coli	40	0.13–1.0	0.25	0.5	Salmon *et al.* (1996)
E. coli	115	0.03–0.25	0.25	0.25	Soback *et al.* (1989)
E. coli	10	0.25	0.25	0.25	Yancey *et al.* (1987)
Arcanobacterium pyogenes	1	≤ 0.06	n.a.	n.a.	Yancey *et al.* (1987)
A. pyogenes	42	0.39–1.56	0.78	1.56	Yoshimura (2000)

n.a. = not available.

The concentrations of ceftiofur would therefore be higher in diseased animals than those reported herein for uterine tissues of normal postpartum cows.

In the current study, the concentrations of ceftiofur observed in plasma, in the uterine tissues and in lochial fluid are above the MIC of the major pathogens identified in acute puerperal metritis during the dosing interval at the dose used.

In conclusion, a single subcutaneous injection of ceftiofur hydrochloride at the dose of 1 mg/kg body weight in healthy postpartum lactating dairy cows produced plasma and endometrial concentrations of ceftiofur that exceed the reported MIC of the most common pathogens involved in acute puerperal metritis, for 24 h.

These results suggest that ceftiofur may be effective *in vivo* for the treatment and control of acute puerperal metritis. The efficacy of ceftiofur has to be proven in field studies.

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