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Araştırma Makalesi ***Research Article***

Effect of Different Intrauterine Oxytetracycline Treatment on Reproductive Performance of Dairy Cows with Clinical Endometritis and Determination of Oxytetracycline Residues in Milk

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Abstract

Main objective of this study was to determine whether intrauterine infusion of oxytetracycline (OTC) is an appropriate method for the treatment of postpartum endometritis in dairy cows or not. To this end, 280 lactating Holstein cows with clinical signs of endometritis were randomly assigned into three treatment groups and 186 cows were sampled for cytology experiment. In group 1 (n=73) cows were treated with intrauterine infusion of 5 g/cow 10% OTC. In group 2 (n=44), before intrauterine OTC treatment, caudal epidural anesthesia was used to eliminate straining. In group 3 (n=49) after intrauterine infusion of 5g/cow 10% OTC, 100 ml of normal saline was injected to reduce the concentration of OTC and 20 untreated cows were assigned into control group (group 4). Clinical treatment rates were 79.4%, 68.1%, 65.3 %and 50% in OTC, OTC and caudal epidural anesthesia (OTC+CEA), OTC+Serum and control groups, respectively (P>0.05). Cytological treatment rate in each experiment group was significantly higher than the cytological treatment rate in control group in the second examination (P<0.05). To find out OTC residues in milk by high performance liquid chromatography (HPLC) method, milk samples of 6 treated cows from each group were collected randomly 12, 24, 48, 72 and 96 hours after treatment. Maximum amounts of OTC residues were found 72 hours after treatment in all treatment groups. To evaluate the reproductive performance, the researchers compared 280 cows of treatment groups were compared with 1088 cows that were clinically healthy without any signs of clinical endometritis. The reproductive performance of the two groups were not significantly different, except days to first service which was lower in cows without clinical endometritis (P<0.05). According to the cytology study and the presence of antibiotic residues in milk samples at different times, the use of intrauterine OTC for treatment of clinical endometritis seems skeptical.

Introduction

Postpartum endometritis is one of the most common uterine disorders in dairy cattle which can reduce reproductive performance of dairy cows (LeBlanc et al., 2002). Therefore, postpartum endometritis can diminish the profitability of dairy operations (Overton and Fetrow, 2008). One of the broad spectrum antibiotics that has been used for the treatment of postpartum endometritis in dairy cows is oxytetracycline (OTC) (Sheldon et al., 2004). After intrauterine injection of OTC, therapeutic concentration will be created on the endometrium and the drug action will be restricted to the uterine lumen and endometrium (Kaczmarowski et al., 2003; Roncada et al., 2000).

Intrauterine OTC can cause direct irritant effect upon the endometrium, stimulate inflammatory response and uterine defensive reactions, and promotes polymorphonuclear leukocytes cell (PMN) infiltration to uterine lumen. It can also lead to the regeneration of uterine tissues (Cohen et al., 1995; Noakes, 2009; Swelum, 2013). Based on clinical observations of the researchers' partners this effect causes strain in the cows and outflow of antibiotic from uterine lumen which could result in reduced drug efficacy and decreased residence time of antibiotic in uterine lumen. Although intrauterine infusion with antibiotics is one of the best protocols for the treatment of postpartum endometritis in dairy cows (Drillich et al., 2005), it can cause drug residues in milk (LeBlanc, 2008).

After intrauterine infusion of OTC, antibiotic residues will be found in milk for 1 to 8 days (Tan et al., 2007) and can usually be detected by a number of biological

methods. Chromatographic technique is a reliable and accurate method to measure the amount of antibiotic residues in milk and meat. High performance liquid chromatography is one of this chromatographic techniques (Fritz and Zuo, 2007). Due to stimulatory effect of OTC on the endometrium and cows straining after intrauterine injection of OTC, the first objective of this study was to find an appropriate method for intrauterine treatment of postpartum endometritis with OTC. After treating dairy cows with antibiotics, drug residues are usually found in milk (Adams, 2001). Due to harmful effects of antibiotic residues in milk for human health, World Health Organization (WHO) has determined maximum residues limit (MRL) in milk for a variety of veterinary practices (Samanidou et al., 2007); the limit is set to 0.1 mg/kg (100 ng/g) in raw cow milk (Navratilova et al., 2009).

The second objective of this study was to investigate the presence and duration of OTC residues in the milk of cows that were treated with three intrauterine administration methods of OTC by HPLC method.

The third objective was to compare the reproductive performance in group of cows with clinical endometritis that were received OTC (group 1, group 2 and group 3) with group of cows without clinical endometritis.

Materials and Methods

Experiment 1

Animals

The study was carried out in a large commercial dairy herd in Iran. Cows were housed in free stall barns and milked three times daily at approximately 8 hour intervals. The average milk production for a fresh cow was 45 kg. Cows in this herd were calved in the clean calving boxes and kept in the individual boxes for at least 10 d after parturition. In total, 280 lactating Holstein cows with clinical signs of endometritis were treated with three methods of intrauterine OTC infusion and cytology samples were obtained from 186 cows. Then, 1088 clinically healthy cows without any signs of clinical endometritis were compared with treatment groups regarding their reproductive performance. These cows did not receive any treatment, at least 14 days before sampling.

Clinical Examination and Sampling

The criteria for selection of cows were as follows: the cows were examined 28-35 days after calving and the cows with mucopurulent vulvar discharge or abnormal uterus at rectal palpation were used for this study. Then, presence of endometritis was confirmed by cytological evaluation of cervical mucosa.

At first, cows were inspected for the presence of fresh discharge around the vulva, perineum, or tail. If discharge was not visible externally, vaginal examination was performed. The cow's vulva was thoroughly cleaned with a dry paper towel and then a clean, lubricated, and gloved hand was inserted through the vulva. In each cow, the lateral, dorsal and ventral walls of the vagina were palpated, and the mucus contents of the vagina were withdrawn manually for examination. The vaginal mucus was assessed in terms of color and the proportion of pus. The vaginal discharge was scored using a 0 to 3 scale, 0=normal uterine discharge, 1=flakes of purulent exudates in the uterine discharge, 2=>50% of the uterine discharge is made up of purulent exudates, 3=hemorrhagic uterine discharge mixed with purulent exudates (Sheldon et al., 2006; Williams et al., 2005). Then, cytological smear was prepared with contacting the slide to discharge for confirming endometritis (Ahmadi et al., 2005a). The smear slides were air-dried, taken to laboratory, fixed with 99.8% methanol, and stained with Giemsa. Then, all slides were examined microscopically for the presence and quantity of phagocytes. Cytological assessment was performed by counting 10 fields at 400× magnification to determine the frequency of neutrophils and epithelial cells (Lu et al., 2011).

The ultrasonographic scanning was performed to assess ovarian structures, diameter of the uterus, echotexture, thickness of the uterine wall and intraluminal fluid accumulation, using a 5 MHz rectal linear probe (Easyscan, BCF, UK). Reproductive tract was also examined by rectal palpation too. Cervical diameter, location of uterus, symmetry of the uterine horns, diameter of (larger) uterine horn, dominant palpable ovarian structure including corpus luteum (CL), follicle, or no palpable structures were recorded (Sheldon et al., 2008).

Treatment Protocols

Cows with clinical endometritis were randomly assigned into one of 4 treatment groups: (1) OTC group: intrauterine infusion of 5 g/cow of 10% OTC (Oxyvet, Razak®, Iran) (Sheldon, 2007) with disposable transcervical pipette (Continental Plastic, SUPA, Iran) performing in the cows (n=73) in this group, (2) OTC+CEA group: due to straining of cows because of the stimulatory effect of OTC and thus outflow of antibiotic, caudal epidural anesthesia was used to eliminate straining in this group of animals (Tranquilli et al., 2013). For this purpose, 2% lidocaine (Vetacaine, Aburaihan®, Iran) was injected at a dose of 0.2 mg/kg in first intercoccygeal space (Vesal et al., 2013). This was located by raising the tail in a 'pump handle' fashion to

identify the first obvious articulation behind the sacrum and anesthesia was confirmed by observing the loss of tail tone (Tranquilli et al., 2013). Then, cows (n=44) were treated by intrauterine 10% OTC (Oxyvet, Razak®, Iran) at a dose of 5 g/cow. (3) OTC+Serum group: After intrauterine infusion of 10% OTC in cows (n=49) of this group, 100 ml of normal saline was injected with disposable transcervical catheter. Intrauterine normal saline reduced the antibiotic concentration in uterus. It is likely that the reduction of OTC concentration reduces the straining of cows. (4) Control group: due to high spontaneous treatment rates in clinical endometritis (Noakes, 2009), untreated cows (n=20) were assigned to this group.

All cows were re-examined 14 days after treatment and examinations and sampling procedures were repeated. Estrus was detected three times daily by a technician and all the animals presenting signs of standing estrous were artificially inseminated by artificial insemination (AI) technician. Pregnancy diagnosis was performed 35-45 days after AI via transrectal ultrasonography and reconfirmation of pregnancy was made approximately 90 days after the AI.

Results of treatment was considered by clinical treatment rate, defined as no vaginal discharge in re-examination after the first treatment and existence of corpus luteum on the ovary. Cytological treatment rate was defined as the reduction in neutrophil numbers in the second examination.

Reproductive performance was determined by measuring days to first service, days open, first service conception rate, conception rate to all services, and cows' pregnancy within 180 days in milk (DIM).

Experiment 2

Milk Sampling

After intrauterine administration of OTC among treatment groups, milk samples of 6 treated cows from each group were collected randomly. Cows were milked by hand from every fourth udder into a sample tube; potassium dichromate was used as a preservative in the tubes (Kroger, 1985).This samples were collected 12, 24, 48, 72, and 96 hours after treatment (Vuković et al., 2011) and stored in the sterilized tubes at -20°C in the refrigerator until assayed. Milk samples were delivered frozen to Food Hygiene Laboratory for HPLC procedure.

Sample Preparation for HPLC Analysis

Before analysis, milk samples were heated to 40 °C, stirred, and cooled to 20°C. Fifteen ml of the each milk sample was mixed with 25 ml of McIlvaine buffer (the buffer was prepared by combining 400 ml 0.2 mol/l sodium hydrogen phosphates, 600 ml 0.1 mol/l of citric acid and 0.37 g of EDTA with pH=4.1). The diluted samples were centrifuged at 4000 rpm for 10 minutes. After centrifugation, the upper fat layer was removed. An aliquot part (25 ml) of the supernatant was subjected to solid phase extraction (SPE) on the Oasis HLB columns, according to the instructions in the user's manual, by the Waters (Milford, USA). SPE columns were conditioned with 3.0 ml of methanol, and washed out with 2.0 ml of water. Then, 25 ml of the supernatant was applied to the column, and washed with 1.5 ml 5% methanol in water. OTC was eluted with 2.0 ml of methanol and then evaporated to dryness on the rotary vacuum evaporator. The evaporated residues were reconstituted in 1.0 ml of the mobile phase and filtered through 0.2 µm nylon filters for HPLC determination. All samples were analyzed right after preparation.

Conditions of HPLC Analysis

Milk samples were analyzed using the liquid chromatography system Breeze (Waters, USA) equipped with a binary gradient pump 1525, dual UV/VIS detector 2487, autosampler 717 plus, and column thermostat. This system was controlled and the data were analyzed using Breeze software. A chromatography column Nova Pack C8, 3.9 mm × 150 mm with the particle size of 4 µm (Waters, USA) was used. To prepare standard solutions, the researchers made the working solution in the concentration range of 0.01–10 mg/l from stock solutions of concentration of approximately 0.1 g/100 ml of OTC hydrochloride O5875 (Sigma Aldrich, Inc., St. Luis, USA) in methanol.

UV detection was carried out at the wavelength of 365 nm. The measurements took place in isocratic mode. The mobile phase (flow rate of 0.8 ml/min) was a mixture of acetonitrile, methanol, and 0.05 mol/l of oxalic acid with the ratio of 13:13:74. The sample injection volume was 20 µl, and the column temperature was 30 °C. External standard method was used for the qualitative and quantitative evaluation. Each sample was analyzed in duplicates at least; every series contained a blank sample. Simultaneously, aliquots of the samples with addition of standard solutions of known concentrations were measured. Detection and quantitation limits were established based on the standard deviation of the blind test and the slopes of the calibration curves. Repeatability was based on 20 parallel determinations and the recovery was based on determinations of the milk sample in addition to the solution of standards of known concentrations (50 µg/l and 100 µg/l).

Statistical Analysis

SPSS software (Version 21) was used for statistical analysis of the data in this study. In the first experiment,

clinical treatment rate in different treatment groups was compared using Chi-square and Fisher's exact tests. One way ANOVA Test was used to evaluate cytological tests. LSD multiple range test was used as a post hoc test to determine significant differences between groups.

In the second experiment, samples with different residues of OTC were compared using GLM procedure. Duncan multiple range test was used as a post hoc test to determine significant differences between groups. Kaplan-Meier survival analysis was performed to produce survival curves of OTC treatment effects on cows' days open. A probability of P<0.05 was used to establish statistical significance.

Results

Classification of endometritis grade in different treatment groups is shown in Table 1.

Clinical treatment rates in this examination (based on the absence of purulent discharge from the vagina in the second examination) were 79.4%, 68.1%, 65.3%, and 50% in OTC, OTC+CEA, OTC+Serum and control group, respectively (P<0.05). Clinical treatment rate was significantly lower in control group than in the OTC and OTC+CEA groups. There was no significant difference between the three treatment groups (P<0.05). These results can be seen in Table 2 and Figure 1.

Considering Figure 2, it can be implied that in all four groups, the number of CLs on ovaries in second examination is higher than number of CLs on ovaries in the first examination, yet there was no significance difference between the three treatment groups in this regard**.**

The results of cytological treatment rate in this experiment (based on the changes in number of neutrophils and epithelial cells in second exam after treatment) are shown in Table 3. Number of neutrophils in each treatment group was significantly lower than the number of neutrophils in the control group in the second examination (P<0.05). There was no significant difference between the mean of neutrophils in OTC group and OTC+CEA group, as well as between OTC+CEA group and OTC+Serum group in second examination. However, the mean of neutrophil numbers in OTC group was significantly lower than their mean in OTC+Serum group in the second examination (P<0.05).

Table 1. Classification of endometritis(Exam 1) in OTC, OTC+CEA, OTC+Serum and Control groups.

Classification	Treatment Groups			
	OTC	**OTC + CEA**	**OTC + Serum**	**Control**
E1 (%)	31 (42.5)	25 (56.8)	17 (34.7)	11 (55)
E2 (%)	26 (35.6)	13 (29.5)	19 (38.8)	5 (25)
E3 (%)	16 (21.9)	6 (13.6)	13 (26.5)	4 (20)
Total (%)	73 (100)	44 (100)	49 (100)	20 (100)

E1=Endometritis 1, E2=Endometritis 2, E3=Endometritis 3.

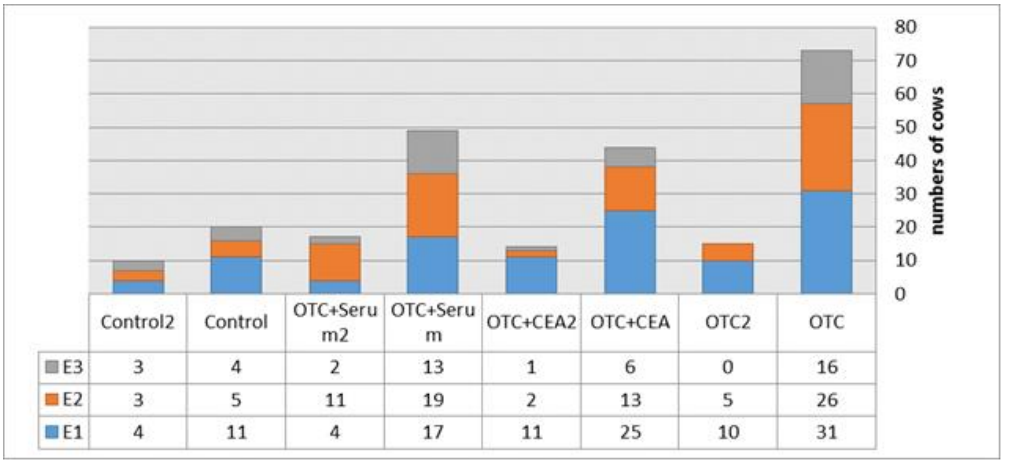

Figure 1. Clinical treatment rate 2 weeks after treatment, OTC, OTC+CEA and OTC + Serum are pre-treatment groups and OTC2, OTC+CEA and OTC + Serum are post-treatment groups.

Table 2. Clinical treatment rate (%) 2 week after first examination of three categories of endometritis in 4 treatment groups.

Treatment Groups	Diagnosis 2		Diagnosis 1			
	Endometritis Categories	No.	Treated Endometritis	E1	E2	E3
OTC	E1	31	24 (77.4)	7 (22.5)	0 (0.0)	0 (0.0)
	E2	26	22 (84.6)	1 (3.8)	3 (11.5)	0 (0.0)
	E3	16	12 (75.0)	2 (12.5)	2 (12.5)	0 (0.0)
	Total	73	58 (79.4)	10 (13.6)	5 (6.8)	0 (0.0)
OTC+CEA	E1	25	15 (60.0)	9 (36.0)	1 (4.0)	0 (0.0)
	E2	13	12 (92.3)	1 (7.6)	0 (0.0)	0 (0.0)
	E3	6	3 (50.0)	1 (16.6)	1 (16.6)	1 (16.6)
	Total	44	30 (68.1)	11 (25.0)	2 (4.5)	1 (2.2)
OTC + Serum	E1	17	10 (58.8)	2 (11.7)	5 (29.4)	0 (0.0)
	E2	19	14 (73.6)	2 (10.5)	2 (10.5)	1 (5.2)
	E3	13	8 (61.5)	0 (0.0)	4 (30.7)	1 (7.6)
	Total	49	32 (65.3)	4 (8.1)	11 (22.4)	2 (4.0)
Control	E1	11	7 (63.6)	3 (27.2)	1 (9.0)	0 (0.0)
	E2	5	2 (40.0)	1 (20.0)	1 (20.0)	1 (20.0)
	E3	4	1 (25.0)	0 (0.0)	1 (25.0)	2 (50.0)
	Total	20	10 (50.0)	4 (20.0)	3 (15.0)	3 (15.0)

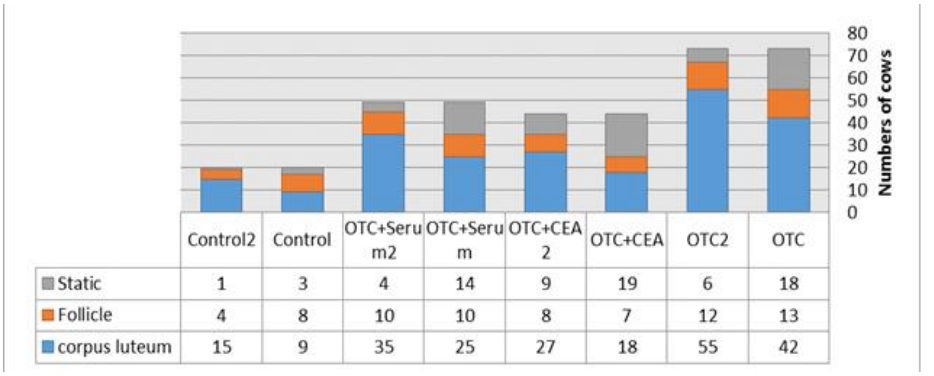

Figure 2. Ovarian structures during treatment and 2 weeks later in cows diagnosed with clinical endometritis.

Table 3. Comparison of cytological change percentages before and 2 weeks after treatment in 4 treatment groups.

Treatment groups	Number	First sample		Second sample	
		Neutrophil	Epithelial Cell	Neutrophil	Epithelial Cell
OTC	73	52.36±17.12	47.63±17.12	15.57±16.08[a]	84.43±15.72
OTC+CEA	44	47.11±19.24	51.75±18.27	20.97±15.85[ab]	77.88±17.09
OTC+Serum	49	55.06±16.48	43.91±15.33	22.97±20.79[b]	76.00±21.41
Control	20	46.20±19.64	53.80±19.64	33.90±29.86[c]	66.10±29.86

[a,b,c] Values with different superscript letters within a column differ significantly at P<0.05.

The results of HPLC analysis are shown in Table 4. All milk samples from each treatment group showed positive results in terms of the presence of antibiotic residues at different times. As shown in Table 4 maximum amounts of OTC residues were found 72 hours after treatment in all treatment groups. The average of antibiotic residues in OTC+CEA group was 189.86±38.42 part per billion (ppB=ng/ml) and it was higher than their average in the other two groups (P<0.05).

In OTC group, antibiotic residues were significantly higher at 72nd hour than at other times after treatment (P<0.05). Samples that were taken at 48th and 72nd hours after treatment had OTC residue levels above the WHO standard (100ng/g).

In OTC + CEA group, antibiotic residues was significantly higher at 72nd hours than at other times after treatment (P<0.05). At 24th, 48th and, 72nd hours, level of antibiotic residues were higher than the WHO standard level.

In OTC + Serum group, antibiotic residues were significantly higher at 48th and 72nd hours than at other times after treatment (P<0.05). Samples that were taken at 24th, 48th and, 72nd hours after treatment had OTC residue levels above the WHO standard (100ng/g).

Levels of antibiotic residues were compared in a specified time between different treatment groups. In 24th hour, the level of antibiotic residues was significantly higher in OTC+Serum group and in 96th hour, it was significantly higher in OTC+CEA group (P<0.05).

Table 4. Results of HPLC analysis for antibiotic residues in milk (ppB=ng/g).

Time	OTC (ppB)		OTC+CEA (ppB)		OTC+Serum (ppB)	
12h	N=5	38±25.44a	N=5	52±12.37a	N=6	60±30.21a
24h	N=6	42.67±26.53aA	N=4	104±20.56aAB	N=5	132±62.95abB
48h	N=6	148.83±40.41b	N=4	192.4±31.23a	N=5	258±77.44b
72h	N=6	276.67±84.89c	N=4	452.4±157.14b	N=6	286.67±78.77b
96h	N=4	12.50±5.05aA	N=4	78±13.09aB	N=6	35±17.55aA
Total	N=27	112.93±28.27	N=21	189.86±38.42	N=28	151.43±30.81

[a,b,c]Values with different superscript letters within a column differ significantly at P<0.05.
[A,B]Values with different superscript letters within a row differ significantly at P<0.05.

To evaluate the reproductive performance, the researchers assigned 280 cows with clinical endometritis into one group; these cows were treated with three methods of intrauterine infusion of OTC which were described above. One thousand eighty eight cows that were clinically healthy without any signs of clinical endometritis were assigned to the other group. There was no significant difference in reproductive performance between two groups except in days to first service which was lower in cows without clinical endometritis (P<0.05). Days open were 136.64±93.25 and 130.60±88.51 in treatment groups and healthy cows, respectively. There was no significant difference in conception rate between treatment groups and the group of cows without clinical endometritis (P≥0.05). Conception rates to all services were 45.01% and 42.98% in treatment groups and cows without endometritis group. This difference was not statistically significant (P≥0.05).

The perecentages of cows that were pregnant within 180 DIM were higher in the group of cows without clinical endometritis, but this difference was not statistically significant (P≥0.05). The reproductive performance data was shown in Table 5.

Figure 3 is a survival curve demonstrating the effect of study's endometritis treatments on pregnancy in cows with endometritis compared with cows without endometritis, in different times. There was no significant difference between OTC treatment groups and healthy cows in the rate of pregnancy (log rank test, P<0.05). About 102 days after parturition, 50% of cows without clinical endometritis became pregnant, but the median days open in treatment groups was 115.

Discussion

A variety of antibiotics have been infused into the uterus to treat postpartum endometritis in dairy cows. OTC is one of the broad spectrum antibiotics that were used for treatment of postpartum endometritis in dairy cows (Ahmadi et al., 2005b; Sheldon et al., 2004). Moreover, because of its effective action in anaerobic environment of the uterus after parturition, OTC is one of the choices for intrauterine treatment of clinical endometritis (Kaczmarowski et al., 2003; Sheldon et al., 2004). In the present study (Experiment 1), for the first time, posterior epidural anesthesia had been used before intrauterine OTC injection, and antibiotic concentration with normal saline was reduced for the treatment of clinical endometritis in dairy cattle. This study showed that all three treatment groups had further proportion of cows that scored 0 (clinical treatment) 14 days after treatment in comparison to control group. Clinical treatment rate was higher in OTC group than in other two treatment groups, but the difference between them was not significant (P≥0.05).

Table 5. Reproductive performance definitions and measures.

Parameter	Definition	Treatment groups (n=280)	Cows without clinical endometritis (n=1088)
Days to first service	Date of first service - Date of calving	86.89±31.88	68.01±19.35
Days open	Date of successful AI - Date of calving	136.64±93.25	130.60±88.51
First service conception rate	Cows pregnant at 1st AI × 100 No. of cows inseminated	125/280 (44.64%)	456/1088 (41.91%)
Conception rate to 2nd and 3rd services	Cows pregnant at 2nd and 3rd AI × 100 No. of cows inseminated in 2nd and 3rd services	109/155 (70.32%)	433/632 (68.51%)
Conception rate to all services	No. of pregnant cows × 100 Total no. of AI	280/622 (45.01%)	1088/2531(42.98%)
Cows pregnant within 180 DIM	No. of cows pregnant within 180 DIM Total no. of inseminated cows	215/280 (76.78%)	864/1088 (79.41%)

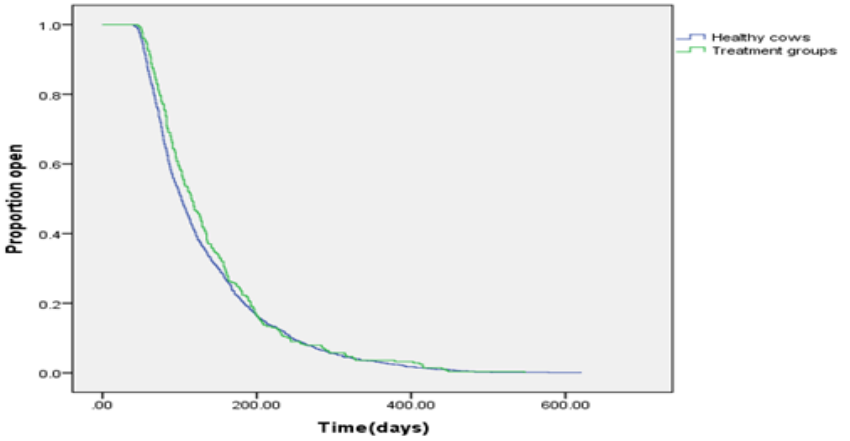

Figure 3. Kaplan-Meier survival curve for proportion of open cows and days open for cows without endometritis and OTC treatment groups.

Treatment groups: n=280; MDO=115 (103, 126) days. Healthy cows group: n=1088; MDO=102 (96, 107) days.

Ahmadi et al (2005) reported that intrauterine infusion of OTC was better than 10 ml of Metri-Care (cephapirin) in treating early stages of postpartum endometritis (Ahmadi et al., 2005b). Although intrauterine OTC does not penetrate the wall of uterus well, it is not particularly effective against T. pyogenes and can have a direct irritant effect on the endometrium (Cohen et al., 1995).

Therefore, OTC can be used as a prophylactic intrauterine antibiotic after assisted calving (Noakes, 2009). Unlike control group in all other three treatment groups, E2 had the best response to treatment. This was consistent with the results of Ahmadi et al (2014). They were observed that cows categorized as E1 and E2 showed higher treatment rate and reproductive performance than E3 cows in hyperimmune serum and OTC treatment groups. However the differences of their statistical analysis were not significant (Ahmadi et al., 2014).

In this study the CL numbers on ovaries was higher in the second examination than the first in all four groups. Proportion of ovary structures (follicles, CL, and cysts) was not different among all four groups which were consistent with the reports of Brick et al. (2012). They reported that neither the proportion of ovarian structures nor cycling status of cows with clinical endometritis differed among intrauterine dextrose and subcutaneous ceftiofur treatment groups (Brick et al., 2012).

In cytological study, the neutrophil numbers in all OTC treatment groups were significantly lower than neutrophil numbers in control group in second examination. This reduction in the neutrophil numbers in OTC group was sharper than in other treatment

groups. Even this amount of neutrophils in OTC group was higher than normal due to the time of sampling (40-45 days after parturition). If the number of neutrophils in uterine smear is above 10% at 34-47 days after parturition, this is an indication of subclinical endometritis (Sheldon et al., 2006). Subclinical endometritis is associated with lower conception rates to first service and overall services (Sheldon et al., 2006).

The widespread use of antimicrobials in dairy cattle management may result in the presence of antibiotic residues in milk. To prevent any harmful health effects on consumers, Food and Agricultural organization (FAO) and European Union (EU) have established a maximum residual limit (MRL) of OTC in milk at 100 ng/ml (Naoto, 1999) and the 'safe level' set by the US Food and Drug Administration is 30 ng/ml for OTC (Papadoyannis et al., 2000).

In this study, the amount of OTC residues was higher than MRL (100 ng/ml) 48 and 72 hours after treatment in all treatment groups. However in OTC+CEA group and OTC+Serum group it was also higher than MRL 24 hours after treatment. Maximum amounts of OTC residues were found 72 hours after treatment which was lower than MRL 12 and 96 hours after treatment in all treatment groups.

After intrauterine application of OTC in a dose of 2.0 g per cow, excretion of residues in milk was monitored using Resazurin test with *Str. thermophilus* and specific quantitative procedure (Vuković et al., 2011). Discontinuation of milk secretion of residues containing OTC was between 34 and 70 hours of experiment. Average discontinuation was 53.2 hours after the injection of the antibiotics. The maximum concentration value of OTC containing residues was at 22nd hour in the range from 1.0 to 2.2 i.e. an average of 1.64 mg / ml. In the following these values decrease and in the 70th hour the average value was 0.08 µg/ml (Vuković et al., 2011). These results were not in line with the results of Kaneene et al. (1986). Namely, after giving intrauterine dose of 3 g OTC, residues could be detected after 12.5 - 44 hours (Kaneene et al., 1986). Similar results were obtained by Parasad et al. (1987) after application of intrauterine dose of 5 mg/kg, they detected residues after 3 and 12 hours (Parasad et al., 1987). Kaale et al. (2008) had developed a rapid reversed phase high performance liquid chromatography method for OTC analysis in raw milk samples. They obtained average recoveries greater than 92% with RSD ranging between 0.8% and 6.6% (Kaale et al., 2008). Sokol et al. (1994) monitored tetracycline antibiotics concentrations in milk after intramammary administration using HPLC (Sokol and Matisova, 1994). When validating HPLC, they obtained a recovery of 95.41% for CTC, 91.98% for OTC, and 83.44% for TTC, and the determination detection limits at 0.05 ppm (50ng/g).

Conclusions

According to the result of cytology and presence of antibiotic residues in milk samples at different times after treatment, the use of intrauterine OTC for the treatment of clinical endometritis seems skeptical. Development of effective alternative therapies instead of antibiotics for the treatment of postpartum endometritis is indispensable and is suggested for further studies.

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