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Preliminary trial in treatment of postpartum endometritis with intrauterine application of hyperimmune serum in dairy cows

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PEER REVIEW

Peer reviewer

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Comments

According to our knowledge, this study is the first report of clinical use of hyperimmune serum for treatment of subclinical endometritis in dairy cows. We suggest that hyperimmunue serum could be the no antibiotic alternative treatment choice for postpartum endometritis in dairy cattle.
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ABSTRACT

Objective: To evaluate the efficacy of hyperimmune serum (HS) in treatment of postpartum endometritis.

Methods: In a field trial, cows with vaginal discharge, 25-35 d in milk were randomly assigned to three treatment groups. In group 1 (*n*=42), cows received an intrauterine treatment with 50 mL HS. HS was produced against *Arcanobacterium pyogenes* and *Escherichia coli* that had already been isolated from the Iranian dairy farms. In group 2 (*n*=39), cows were treated with one intrauterine infusion of 5 g oxytetracycline (OTC). In group 3 (*n*=65), cows affected with endometritis were treated with 0.5 mg cloprostenol (PG). Group 4 (*n*=89) included clinically healthy cows as control (HC) group without any treatment in groups HS, OTC and PG; all cows were re-examined 39-49 d in milk.

Reults: Cure rate after treatment defined as the absence of vaginal discharge at the re-examination, was 64.3%, 61.5% and 72.3% in HS, OTC and PG groups, respectively (*P*>0.05). Cows categorized as E1 and E2 showed higher cure rate and reproductive performance measures than E3 cows in both treatment groups but their differences were not significant. Conception rate to all services for cows with endometritis (category E1, E2 and E3) was 52.9% in HS group, 57.1% in OTC and in PG 62.1% compared to 66.7% in HC (*P*>0.05). The difference in the conception rate between the HS and HC group was not significant.

Conclusions: We suggest that HS could be the no antibiotic alternative treatment choice for postpartum endometritis in dairy cattle.

KEYWORDS

1. Introduction

The uterus is routinely contaminated with bacteria in the early postpartum period; vast majority of cows have bacterial contamination of the uterus in the first 2 to 3 weeks postpartum[1]. Many of these bacteria waste during the first 5 weeks after parturition, but the remaining infection causes uterine disease in some cows[2]. These bacterial infections cause histological lesions and inflammation of the endometrium and delay in uterine involution[3]. The continued bacterial contamination of postpartum uterus causes inflammation, pathological changes of the endometrium and delayed uterine involution[4]. Therefore, uterine diseases must be identified and treated efficiently and timely in order to decrease their negative effects on fertility[1]. There are many complications in pathogenic factors of endometritis. It is suggested that pathogenic bacteria have an important role

in occurrence of endometritis[5]. Infections with *Escherichia coli* (*E. coli*) and *Trueperella pyogenes* (*T. pyogenes*) are associated with both metritis and postpartum vaginal discharge. In the early postpartum period, infections of the uterus with *E. coli* pave the way for subsequent infection with other bacteria[6]. The most prevalent bacteria in the late postpartum period are *T. pyogenes*[7,8]. When *T. pyogenes* was isolated from uterine fluids approximately 21 d postpartum, cows developed severe endometritis and were usually infertile at first service[9]. *T. pyogenes* were strongly associated with clinical endometritis when detected at the 34 to 36 d postpartum[9]. Bicalho *et al.* found *E. coli* at 34 to 36 d postpartum was not associated with clinical endometritis or reproductive failure[8].

The regulation of endometrial immunity depends on steroid hormones, somatotrophins, and possibly local regulatory proteins such as galectins. Advances in knowledge about infection and immunity in the female genital tract should be exploited to develop new treatments and prevention strategies for uterine disease[10]. There are several uterine defense mechanisms against bacterial contamination. The initial uterine defense mechanism against bacterial infection is phagocytosis by uterine leucocytes (mainly neutrophils). The abnormal puerperium results in adverse effects on uterine defense mechanisms and prolongs the time to complete uterine involution. Immunoglobulins have been found in bovine uterine secretions and their protective role against pathogens has been widely reported[1]. Asbury *et al.* (1984) demonstrated that the addition of a small amount of serum to uterine secretions can enhance the opsonizing capacity and significantly increase the phagocytic capacity of equine polymorphonuclear cells. In another study, mares with subclinical endometritis that were treated with a single intrauterine infusion of plasma after mating became preganant[11].

There are no reports on immune serum and opsonizition mechanism treatment of postpartum endometritis in dairy cows. So the objectives of the present study were: firstly to produce hyperimmune serum (HS) as an intrauterine therapy for treatment of postpartum endometritis in dairy cows; secondly to evaluate and compare the response to treatments (clinical cure 14 d post therapy) in lactating dairy cows diagnosed with clinical endometritis, and finally to compare the reproductive performance after intrauterine infusion of hyperimmune serum as a no antibiotic therapy of endometritis affected cows.

2. Materials and methods

2.1. Animals

The study was carried out in a large commercial dairy herd of Iran. Two hundred and thirty five postpartum dairy cows were examined once between 25 and 35 d postpartum. These cows did not receive intrauterine treatment after parturition. In total, 146 clinical endometritis affected cows were selected. Cows in this herd were calved in calving boxes hygienically and kept in individual boxes for at least 10 d after parturition. Corn silage, alfalfa hay, and concentrates as a total mixed ration were used. None of the cows received any intrauterine or reproductive hormonal therapy at least 10 d before sampling for this study.

2.2. HS production

HSs were produced against *Arcanobacterium pyogenes* and *E. coli* that had already been isolated as the most common pathogen bacteria isolates from cases of endometritis in the Iranian dairy farms[7]. Antigens were prepared via sonication of soy broth cultured bacteria pellets. Two healthy heifers were injected subcutaneously with each bacterial antigen at two-week intervals. Antibodies against these bacteria were detected in serum and reached a peak value after 8 injections. Blood collection and serum preparation were carried out in a large amount.

2.3. Clinical examination

At first, cows were inspected for the presence of fresh discharge on the vulva, perineum, or tail. If discharge was not visible externally, cows were examined vaginally. 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 for color and proportion of pus. Endometritis was classified in three categories: clear mucus with flakes of pus (E1), mucopurulent discharge or fluctuating contents in the uterus (E2), and purulent discharge with or without palpable contents in the uterus (E3). Ultrasonographic assessment of uterus and ovaries using a 5 MHz rectal linear probe (AMI Company, Canada) was also performed. Diameter of the uterus, echotexture and thickness of the uterine wall and intraluminal fluid accumulation were evaluated in the cows. Ovarian structures [follicle and corpus luteum (CL)] were scanned. Following inspection, transrectal palpation of the reproductive tract was performed and cervical diameter, location of the uterus, symmetry of the uterine horns, diameter of the (larger) uterine horn, texture of uterine wall, palpable uterine lumen, dominant palpable ovarian structure including corpus luteum (CL), follicle, or no palpable structures were recorded[12].

2.4. Treatment protocols

At first examination [25–35 d in milk (DIM)], all

endometritis infected cows were randomly assigned to receive one of the three treatments: prepared HS (25 mL intrauterine HS mixed with 25 mL sterile 1x PBS iu), 5 g of oxytetracycline (OTC) (Razak®, Iran) and in cows with CL, 500 mg of cloprostenol (PG) (EstroPlan®–UK). Cows with no clinical signs of endometritis were regarded as healthy control group (HC, n=89) and received no treatment. Since blinding was not possible due to the route type of administration, no placebo was used for the control group. All treatments were administered to the cows by the investigating clinician. In each treatment group, all cows were re−examined 14 d later (39–49 DIM) by rectal palpation of the genital tract. The voluntary waiting period was set at 50 DIM. Cows were artificially inseminated on observed estrus by an AI technician. Pregnancy diagnosis was performed 50–60 d after AI. The outcome of the treatment was assessed by clinical cure rate, defined as no vaginal discharge at the re−examination after the first treatment, and reproductive performance measures in the current lactation. Reproductive performance measures and their definitions are described in Table 1.

Table 1

Definitions of reproductive parameters for assessment of the outcome of the treatment in dairy cows.

Parameter	Definition
Days to first service	Date of first service − Date of calving
Days open	Date of successful AI − Date of calving
First service conception rate	Cows pregnant at 1st AI × 100
Conception rate to 2nd and 3rd services	No. of cows inseminated
	Cows pregnant at 2nd and 3rd AI × 100
Conception rate to all services	No. of cows inseminated in 2nd and 3rd services
	No. of pregnant cows × 100
Cows pregnant within 180 DIM	Total no. of AI
	No. of cows pregnant within 180 DIM
	Total no. of inseminated cows

2.5. Statistical analysis

Data were analyzed by using SAS software, (Version 9.1). Days to first service and days open of endometritis cows with different discharges were compared with one way ANOVA. Duncan multiple range test was used as a post hoc test for determination of significant difference between groups. Clinical cure rate, conception rate, first service conception rate, conception rate to further services, conception rate to all services, and pregnant cows were compared using Chi−square and Fisher's exact tests. The crude estimate of the treatment effect on median days to first service and median days open was determined by Kaplan−Meier Survival Analysis. Graphs of cumulative pregnancy risk over time were generated using spline curve in SigmaPlot (Version 5.0, SPSS Inc., 1999). The level of significance was considered at $P<0.05$.

3. Results

A total of 235 cows were randomly selected in the study.

Cows with asymmetrical uterine horns and vaginal discharge 25−35 DIM (n=146) were randomly assigned to three treatment groups: HS treated group (n=42), OTC treated group (n=39) and PG treated group (n=65). The classification of endometritis at Exam 1 in different treatment groups is shown in Table 2.

There was no cow with clinical side effects after intrauterine infusion of HS. Clinical cure rate after the first treatment, defined as the absence of vaginal discharge at the second examination, was 64.3%, 61.5% and 72.3% in HS, OTC and PG groups, respectively ($P>0.05$). The differences in cure rates for all cows and for categories of endometritis (E1 to E3) at Exam 1 were statistically not significant between the groups. Diagnosis results at Exam 2 are shown in Table 3.

Table 2

Classification of endometritis at 25−35 DIM (Exam 1) in HS, OTC and PG groups.

Classification	Treatment groups		
	HS (n=42)	OTC (n=39)	PG (n=65)
E1 (%)	25 (59.5)	20 (51.3)	61 (93.8)
E2 (%)	12 (28.6)	12 (30.8)	4 (6.2)
E3 (%)	5 (11.9)	7 (17.9)	0 (0.0)
Total (%)	42 (100)	39 (100)	65 (100)

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

Table 3

Cure rate after the first treatment at 39−49 DIM (Exam 2) for three categories of endometritis in different treatment groups.

Treatment groups	Diagnosis in exam 1		Diagnosis in exam 2 n (%)			
	Endometritis categories	No.	Treated endometritis	E1	E2	E3
HS	E1	25	18 (72.0)	6 (24.0)	0 (0.0)	1 (4.0)
	E2	12	7 (58.3)	3 (25.0)	2 (16.7)	0 (0.0)
	E3	5	2 (40.0)	3 (60.0)	0 (0.0)	0 (0.0)
	Total	42	27 (64.3)	12 (28.6)	2 (4.8)	1 (2.4)
OTC	E1	20	13 (65.0)	5 (25.0)	2 (10.0)	0 (0.0)
	E2	12	7 (58.3)	4 (33.3)	1 (8.3)	0 (0.0)
	E3	7	4 (57.1)	2 (28.6)	1 (14.3)	0 (0.0)
	Total	39	24 (61.5)	11 (28.2)	4 (10.3)	0 (0.0)
PG	E1	61	44 (72.13)	15 (24.6)	1 (1.6)	1(1.6)
	E2	4	3 (75.0)	1 (25.0)	0 (0.0)	0 (0.0)
	E3	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Total	65	47 (72.3)	17 (26.2)	1 (1.5)	1 (1.5)

After Exam 2, two cows in OTC group, one cow in PG group and two cows in HC group were withdrawn from the study because of culling decision, so their data was not included in the statistical data analysis of reproductive performance measures. Reproductive performance measures except days open showed no significant differences ($P>0.05$) between the treatment groups (Table 4). There was no significant difference ($P>0.05$) in conception rate between HS, OTC and PG treatments groups compared to HC. Conception rates to all services and pregnant cows percentages by 180 DIM were 90.5%, 86.5%, 90.8% and 92.1% in HS, OTC, PG and HC groups, respectively ($P>0.05$). In HS and OTC treatment groups, cure

rate and reproductive performance measures were higher for cows categorized as E1 or E2 than for cows categorized as E3, but their differences were not significant. Conception rate to all services for cows with endometritis (category E1, E2 and E3) was 52.9% in HS group, 57.1% in OTC group and 62.1% in PG group compared to 66.7% in HC (P>0.05).

Table 4

Reproductive performance measures for groups HS, OTC, PG and HC, respectively.

Parameter	Treatment groups			
	HS (n=42)	OTC (n=39)	PG (n=65)	HC (n=89)
Days to first service*	77.9±27.3	80.9±21.4	73.8±20.9	73.3±24.7
Days open *	95.9±39.8[a]	89.0±29.9[a]	84.2±28.9[a]	78.7±30.5[b]
First service conception rate	23/42 (54.8%)	23/37 (62.2%)	44/65 (67.7%)	66/89 (74.2%)
Conception rate to 2nd and 3rd services	13/26 (50.0%)	9/19 (47.4%)	15/32 (46.9%)	16/34 (47.1%)
Conception rate to all services	36/68 (52.9%)	32/56 (57.1%)	59/95 (62.1%)	82/123 (66.7%)
Cows pregnant within 180 DIM	38/42 (90.5%)	32/37 (86.5%)	59/65 (90.8%)	82/89 (92.1%)

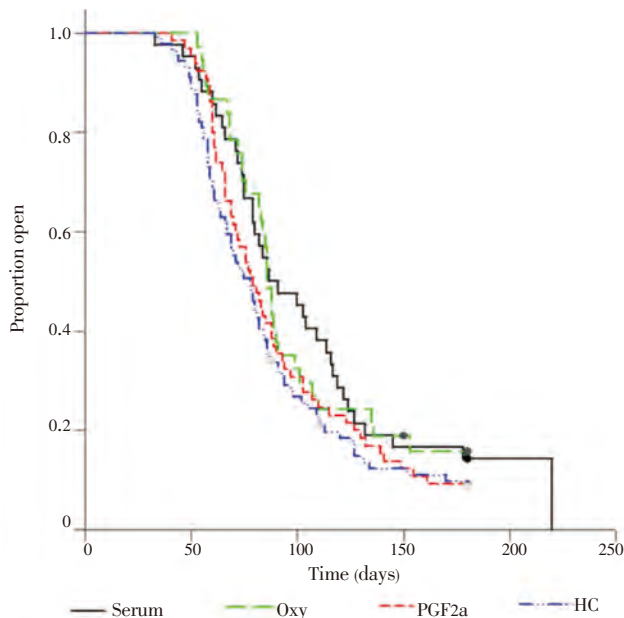Values in rows with different letters (a, b) differ significantly (P<0.05). * mean±SD

Figure 1. Kaplan–Meier survival curve for proportion of open cows and days open for postpartum endometritis cows treated between 25 and 35 DIM with HS, OTC, PG or HC. (——) HS: n=42; MDO=87 (72, 124) days; 12% censored. (— —) OTC: n=39; MDO=86 (74, 110) days; 16% censored. (– – –) PG: n=65; MDO=79(62, 110) days; 9% censored. (–●●–●●) HC: n=89; MDO=78(58, 105) days; 10% censored.

Figure 1 is a survival curve demonstrating the effect of our endometritis treatments on time to pregnancy in endometritis affected compared to HC cows. There was no difference in the rate at which three treatment group cows became pregnant compared to control cows (log rank test, P>0.05). About 78 d postpartum, 50% of HC cows became pregnant, but the median days open in HS, OTC, and PG groups was 87, 86 and 79, respectively (P>0.05). The proportion of censored values for groups HS, OTC, PG and control were not different (P>0.05). Times bred for 50% of cows to become pregnant were 1 for all treatment groups, which was not significantly different from HC group (P>0.05). Means of services per conception for HS, OTC, PG and control groups were not significantly different (1.52, 1.38, 1.32 and

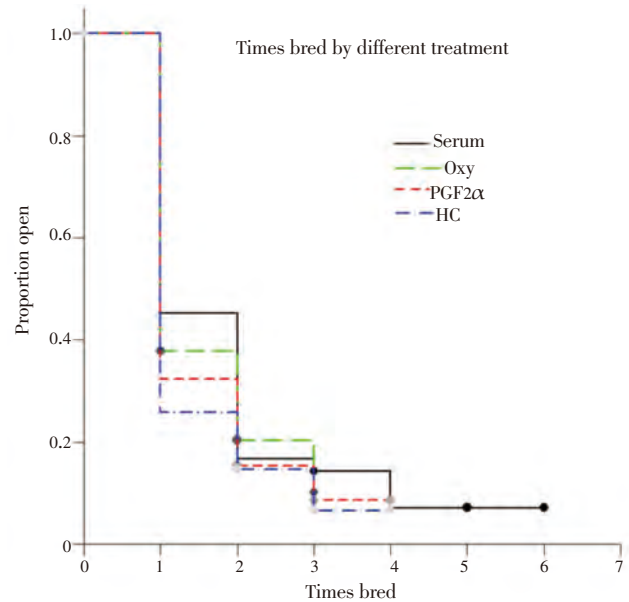1.26, respectively) (P>0.05) (Figure 2).

Figure 2. Survival analysis of times bred. Kaplan–Meier survival curve for times bred for postpartum endometritis cows treated between 25 and 35 DIM with HS, OTC, PG or HC. (——) HS: n=42; Median times bred=1 (1, 2) times; 4% censored. (— —) OTC: n=39; Median times bred =1 (1, 2) times; 7% censored. (– – –) PG: n=65; Median times bred =1 (1, 2) times; 7% censored. (–●●–●●) HC: n=89; Median times bred =1 (1, 2) times; 8% censored.

4. Discussion

The result of this study revealed that the intrauterine infusion of HS was safe in treatment of postpartum clinical endometritis affected cows. Our result showed the acceptable clinical cure rate after treatment of the clinical endometritis affected cows by HS compared to OTC and PG groups. The result of our study revealed similar conception in 4 groups, HS, OTC and PG, compared to HC. Therefore, the treatment result was acceptable and HS showed successful result in endometritis affected cows. A variety of antimicrobial agents, administered by intrauterine infusion or parenteral injection, are used to treat uterine infections[13,14]. The bacteria *Arcanobacterium pyogenes* followed by *Streptococcus* spp. and *E. coli* are more commonly isolated in clinical endometritis in cows and the drugs cefoxitin and sulphamethaxazole are highly effective[15]. The cellular immune response in the uterus may be adversely affected by several therapeutic strategies, such as intrauterine administration of antiseptics, disinfectants and antibiotics, which are commonly used to treat postpartum disorders in cattle[4].

Normal and active defense mechanisms of reproductive system are very important for the uterine elimination of bacterial infection and recovery from endometritis developing after parturition. Local or systemic antibiotics, antiseptics, sulfonamides and hormones are commonly used in practice, but rates of recovery from endometritis and the cow's subsequent fertility have not increased appreciably. Furthermore, the cost of any treatment, the frequency of its

administration and the milk disposal after treatment make their use uneconomic. So Hussein and Daniel suggested application of serum, plasma or HS as an alternative therapy for treatments of bovine endometritis which stimulate the natural uterine defense mechanisms. According to our knowledge, this study is the first report of clinical use of HS for treatment of subclinical endometritis in dairy cows[16].

For the treatment of chronic endometritis in dairy cows, the use of prostaglandin F2a is established in veterinary routine therapy[17]. The administration of PGF2α is often recommended to treat clinical endometritis; conflicting data exist in the literature regarding the potential reproductive benefits[18,19]. Also, an intrauterine treatment with antibiotics has been found effective[13]. The intrauterine treatment with disinfectants, *e.g.* Lugols iodine is widely used in Europe, though several studies have shown no benefits from this treatment on reproductive performance[17].

The findings of Lu *et al.* indicate that liquid paraffin stimulates phagocytic migration into the uterine lumen in cows and that liquid paraffin infusion into the uterus might enhance uterine defense mechanisms during uterine infection[20]. The use of intrauterine dextrose in cows with clinical endometritis may favor a quicker uterine recovery by inhibiting bacterial growth locally, increasing uterine tone, or by nurturing endometrial cells compared with control animals or ceftiofur crystalline free acid cows[21]. An ideal treatment of endometritis should eliminate bacteria in the uterine cavity and, in the sub endometrial layers, not inhibit the normal uterine defense mechanisms, and have no withdrawal period for milk[13]. However, in public and political opinion, the use of antibiotics and hormones in food–producing animals is increasingly under critical discussion. Currently, in the USA, there is no approved antibiotic for intrauterine administration in dairy cows[22]. The result of this study revealed that HS could be an alternative no antibiotic therapy in the cows affected by postpartum endometritis. Drillich *et al.* (2005) used the proteolytic enzymes for treatment of bovine chronic endometritis in a field trial in comparison with a repeated treatment with prostaglandin F2a–analogue cloprostenol with a 14–day interval. The treatment of chronic endometritis with enzymes had never been evaluated in a controlled field study before[23].

It is shown that the decreased phagocytic capacity of leukocytes during the peripartum period, including at the prepartum time point, makes cows more susceptible to postpartum endometritis[24]. Immunomodulators of the uterine defense mechanisms are an alternative to the usual treatment of intrauterine antibiotics, antiseptics, and disinfectants. The administration of single infusion intrauterine *E. coli* lipopolysaccharide as an immunomodulator during estrus, as in cows with bacterial endometritis, stimulated uterine defense mechanism and cleared the infection within one estrous cycle, thereby restoring fertility[25]. This demonstrates that the clearance of uterine lumen with non–invasive methods, such as the use of prostaglandins in the luteal phase and uterine lavage

with normal saline, may have positive effects on the fertility of repeat breeder cows[26].

In conclusion, treatment of endometritis cows with HS was not different from treatment with oxytetracycline and prostaglandin. However, conception rate of these treatments was similar to HC cows that show positive effects of all these treatments on reproductive performance of postpartum cows. It is our opinion that this subject requires further study. Therefore, we suggest that HS could be the no antibiotic alternative treatment choice for postpartum endometritis in dairy cattle. To ensure treatment, we propose the production of the HS anti more bacteria in future.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Comments

Background

Immunoglobulins have been found in bovine uterine secretions and their protective role against pathogens has been widely reported. Asbury *et al.* (1984) demonstrated that the addition of a small amount of serum to uterine secretions can enhance the opsonizing capacity and significantly increase the phagosytic capacity of equine polymorphonuclear cells.

Research frontiers

There are no reports on immune serum and opsonizition mechanism treatment of postpartum endometritis in dairy cows. So the objectives of the present study were: firstly to produce hyperimmune serum as an intrauterine therapy for treatment of postpartum endometritis in dairy cows; secondly to evaluate and compare the response to treatments in lactating dairy cows.

Related reports

It suggested that application of serum, plasma or hyperimmune serum as an alternative therapy for treatments of bovine endometritis which stimulate the natural uterine defense mechanisms.

Innovations & breakthroughs

There was no cow with clinical side effects after intrauterine infusion of hyperimmune serum. Clinical

cure rate after the first treatment, defined as the absence of vaginal discharge at the second examination, was 64.3%, 61.5% and 72.3% in HS, OTC and PG groups, respectively ($P>0.05$).

Applications

It is our opinion that this subject requires further study. Hyperimmune serum could be the no antibiotic alternative treatment choice for postpartum endometritis and post service endometritis should be in chronic endometritis in dairy cattle.

Peer review

According to our knowledge, this study is the first report of clinical use of hyperimmune serum for treatment of subclinical endometritis in dairy cows. We suggest that hyperimmunue serum could be the no antibiotic alternative treatment choice for postpartum endometritis in dairy cattle.

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