

## **Original Article**

# **The effect of ceftiofur hydrochloride treatment in acute *Escherichia coli* mastitis in dairy cattle: a randomized clinical trial**

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## **Summary**

Mastitis caused by *Escherichia coli* (*E. coli*) is one of the most common causes of environmental mastitis in dairy cows, which can impact on the milk quality. In this randomized clinical trial, dairy cattle with naturally acquired acute *E. coli* mastitis (between January 2014 and December 2016, n = 100) were treated with ceftiofur hydrochloride (group A) or placebo (group B) and their rates of clinical recovery 5 days after starting treatment were compared. The cows were randomized into two groups with a 1:1 allocation ratio on the basis of their ear tag numbers. All cows concurrently received supportive measures such as anti-inflammatory treatment, fluid therapy, and frequent milking. Of the 50 cows in the antibiotic group, 41 (82%) recovered clinically. Of the 50 cows in the placebo group, two (4%) recovered ( $P < 0.01$ ). The rates of quarter recovery in the two groups on day 5 were 31.7% and 0%, respectively ( $P < 0.01$ ). Our results suggest that even when treated with intensive supportive therapy, cows with naturally acquired acute *E. coli* mastitis will exhibit poor recovery rates if they are not treated with an effective antibiotic such as ceftiofur hydrochloride. Regarding the presence and shedding of the *E. coli* microorganisms in the milk, it should be considered in relation to public health.

**Keywords:** Dairy cow, Acute mastitis, *Escherichia coli*, Treatment, Ceftiofur

## **Introduction**

*Escherichia coli* (*E. coli*) infection in dairy cows is considered as one of the most common

causes of environmental mastitis in different countries. The bacterium is a ubiquitous inhabitant of soil, the digestive tract, feces and

leads to mastitis in cattle by multiplying in contaminated bedding and entering the mammary glands via the end of the teat when the teat comes in contact with it (Persson et al., 2015). Once inside the mammary gland, the bacterium reproduces rapidly. Then, the immune system of the cow lyses the bacteria, which causes release of endotoxins into the cow circulatory system. Endotoxins (also known as lipopolysaccharide) are complexes of lipid and polysaccharide that are located in the outer membrane of Gram-negative bacteria such as *E. coli*. The lipid portion of the complex, once released into the blood stream, acts as an endotoxin (Persson et al., 2015). Of the cows that naturally acquire coliform mastitis, 32%–75% develop septicemia along with the characteristic clinical signs of *E. coli* endotoxemia, which include loss of appetite, mammary gland inflammation, watery and serum-like milk, high fever, diarrhea, dehydration, and recumbency (Katholm and Anderson, 1992). Toxemia results in dehydration and disseminated intravascular coagulation (DIC), which can lead to mortality of untreated animals in a few days (Blowey and Edmondson, 2010).

Anderson (1989) suggests that administration of antibiotics, anti-inflammatory agents, and fluids may be a beneficial treatment due to endotoxin-induced shock. However, the effect of such treatment protocols on the course of Gram-negative mastitis remains relatively poorly researched to date. A few studies have assessed on the effects of antibiotic-based treatment protocols, including that by Hagiwara et al. (2014). In the mentioned study, which was conducted in Japan, compared antibiotic-treated cows with acute *E. coli* mastitis that did and did not die: they showed that death was related to dysstasia, reduced antithrombin activity, reduced platelet counts, and enhanced hematocrit values and non-esterified fatty acid concentrations. In this regard, it was also suggested that when cows with *E. coli* mastitis were treated with placebo and enrofloxacin, the enrofloxacin-treated cows had lower somatic cell counts in their milk than placebo-treated cows (Persson et al., 2015). However, this treatment did not increase survival compared with placebo. In addition, it was found that in cases of spontaneous and induced coliform mastitis, an application of the intra-mammary antibiotics that are impressive *in vitro* does not ameliorate

the cure rate or alter the elimination of bacteria from the mammary gland (Pyorala, 2009; Hogan and Smith, 2003). However, other studies showed that both acute and mild *E. coli* mastitis can be accompanied by bacteremia; they because cows are physiologically immunosuppressed during the peripartum period, those with acute coliform mastitis may benefit from parenteral antimicrobial treatment (Cebra et al., 1996; Wenz et al., 2001).

To improve our understanding of the efficacy of antibiotic treatment in dairy cows with *E. coli* mastitis, we performed a randomized clinical trial. Thus, dairy cows with naturally occurring acute *E. coli* mastitis were treated with supportive measures together with ceftiofur hydrochloride and placebo, and their clinical recovery assessed 5 days after commencing treatment.

## Materials and methods

### *Animals*

This randomized clinical trial, conducted between January 2014 and December 2016, involved 36 herds of Holstein-Friesian dairy cows located in Tabriz (38° 07' N and 46° 29' E), East-Azerbaijan Province, Iran. During the

trial, the ambient temperature extended from -10°C to 38°C. Of note, the annual rainfall in this city varies from 226 mm to 250 mm. All animals were kept in open shed barns and fed hay, corn silage, soybean, and complementary minerals. They were milked three times a day and their mean production was 30 kg milk/day/cow.

All affected cows had clinical signs of acute mastitis, including a high fever ( $\geq 40^{\circ}\text{C}$ ), redness and inflammation of the mammary gland, watery and yellowish milk, diarrhea, dehydration, and recumbency. The history of the affected cows was recorded, which included the date of mastitis onset, the parity, the bed type, the diet formulation, and the date of recent parturition or pregnancy.

### *Sampling*

All cows that were naturally affected by acute clinical *E. coli* mastitis ( $n = 100$ ) during the study period were considered for inclusion in the trial. Of note, before a treatment, milk samples were also obtained from the affected quarters into sterile culture tubes, according to National Mastitis Council (NMC) guidelines and standards (Hogan, 2017). All samples were subjected to bacterial culture (Harmon et al., 1990) in the Tabriz University

Microbiology Laboratory. After collecting milk samples and before the laboratory results were recorded, all affected cows were administered supportive therapy. Moreover, the cows with odd-numbered ear tags (group A) were administered ceftiofur hydrochloride (Maymó Labs., Spain). The cows with even-numbered ear tags (group B) received placebo instead of antibiotic.

#### *Clinical treatment*

After receiving the laboratory data, all cows (n=100) with acute *E. coli* mastitis were enrolled in the trial. The remaining cows (264 cases), which had clinical mastitis due to other causes (e.g., *Streptococcus uberis*, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*), were excluded from the trial.

Supportive therapy comprised intramuscular flunixin meglumine (Razak Labs. Co. Iran) 2.2 mg/kg bodyweight/day for 4 days and, on the first day of treatment, a single intravenous dose of hypertonic saline (Zoopha Parnian Pars, Iran) (NaCl, 7.2 %; 5 ml/kg bodyweight) and calcium borogluconate 40% (Nasr Company, Iran) (250–500 ml/cow). Antibiotic treatment comprised daily intramuscular injections of ceftiofur hydrochloride (1 mg/5 kg

bodyweight) into the neck region for 4 days. Placebo treatment consisted of daily intramuscular injections of saline into the neck region for 4 days. The cows in both groups were allowed water *ad libitum*, and the affected quarters were milked out every 2 hours to reduce the toxic effects of *E. coli* mastitis. All animals were evaluated daily and heart rate, appetite, body temperature, and color change in the milk from the affected quarters were noted. Additionally, on the 5<sup>th</sup> day of treatment, all surviving cows were examined clinically.

#### *Statistical analyses*

The cows with (Group A, n = 50 cows) and without (Group B, n = 50 cows) antibiotic treatment were compared in terms of primary and secondary outcomes using Chi-squared tests. All statistical tests were conducted using SPSS software (version 22, Inc., Chicago, IL, USA). Differences were considered significant when  $P < 0.05$ .

## **Results**

In total, 364 cows developed acute mastitis during the study period. In 264 cases, mastitis

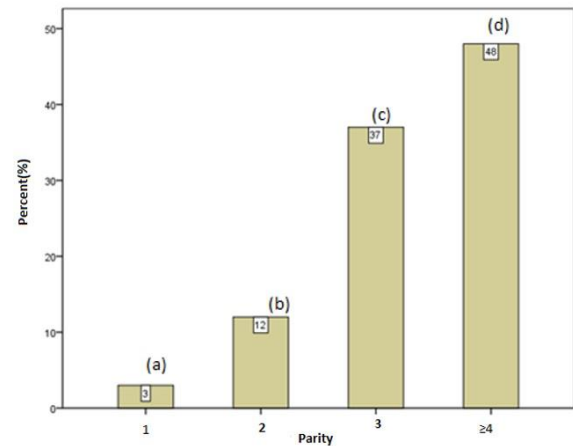
was caused by bacteria other than *E. coli*. These cows were excluded from the study.

The primary outcome of the trial was the clinical recovery of the animal on day 5 of treatment. This was defined as normal clinical signs, including body temperature, appetite, heart rate, and respiratory rate. The secondary trial outcome was quarter recovery on day 5; namely, affected quarters that produced milk with a normal color and consistency.

Of the 100 cows with *E. coli* mastitis, three (3%) were heifers. The remaining 97 were multiparous cows with second (12%), third (37%), and  $\geq$  fourth (48%) parity. The two groups differed in terms of the distribution of parity ( $P < 0.01$ ) (Fig. 1). The most common clinical signs in both groups were recumbency and serum-like milk. The two groups did not differ in terms of clinical sign rates.

We assessed how well the cow history factors associated with *E. coli* mastitis. Of the various factors that were recorded, the type of cow bed was particularly important: cows with dried manure solids beds were more likely to develop *E. coli* mastitis (52%), followed by cows with saw dust beds (39%) and cows with sand beds (free-stall barns) (9%). The two

groups differed significantly in terms of the distribution of bed type ( $P < 0.01$ ).



**Fig. 1.** Parity distribution in dairy cows with *Escherichia coli* mastitis. The two groups differed significantly in terms of parity distribution ( $P < 0.01$ , as determined by the Chi-squared test).

The cows that received antibiotics, were significantly more likely to recover clinically (41/50 cows, 82%) than the cows that received placebo (2/50, 4%;  $P < 0.01$ ) (Table 1). All cows in the two groups that did not recover were slaughtered because of the severity of disease and/or failure of the treatment to meliorate their condition from recumbency to standing.

The affected quarters of the antibiotic-treated cows were also significantly more likely to recover (13/41, 31.7%) than the quarters of the placebo-treated cows (0/2, 0%)  $P < 0.01$  (Table 1).

**Table 1.** Rates of clinical and quarter recovery in dairy cows treated with ceftiofur hydrochloride or placebo ( $P < 0.01$ ).

Group	Clinical recovery of treated cows	Quarter recovery rate
Ceftiofur hydrochloride (A)	41/50 (82%)	13/41 (31.7%)
Placebo (B)	2/50 (4%)	0/2 (0%)

## Discussion

*E. coli* mastitis in cattle can vary from a subclinical mastitis to an acute lethal disease characterized by significant clinical symptoms. Therefore, it can lead to a considerable economic loss in modern dairy farms. Since both subacute and acute *E. coli* mastitis can be accompanied by bacteremia (Cebra et al., 1996; Wenz et al., 2001), parenteral antimicrobial therapy, which is administered simultaneously with fluid therapy, is likely to be beneficial for animals with acute *E. coli* mastitis. This notion is supported in particular by the fact that the postpartum period associated with physiological immunosuppression in dairy cows. However, it remains unclear how effective local antimicrobial agents are for treating *E. coli* mastitis (Suojala et al., 2013;

Hogan and Smith, 2003). In particular, growing evidence show that when enrofloxacin is used to treat acute clinical *E. coli* mastitis, the treatment does not increase survival relative to placebo (Persson et al., 2015; Bargi et al., 2015).

Our study showed that parenteral use of an effective antibacterial agent such as ceftiofur hydrochloride effectively increased the clinical recovery of naturally affected cows: 82% of the antibiotic-treated cows recovered, as opposed to 4% in the placebo-treated group. This finding is in agreement with a previous study which proposed that ceftiofur therapy reduces the mortality rates of dairy cattle with severe clinical mastitis caused by coliform organisms (Erskine et al., 2002). Ceftiofur is a third-generation cephalosporin that is impressive against a wide range of Gram-negative and Gram-positive bacterial pathogens that cause mastitis (Truchetti et al.,

2014). Our results also agree with those of Suojala et al. (2013), who proposed that acute *E. coli* mastitis due to bacteremia following the multiplication of bacteria in the mammary gland should be treated with fluoroquinolones or third- or fourth-generation cephalosporins.

In the current study, both antibiotic- and placebo-treated cows were treated with supportive therapy; namely, fluid therapy, anti-inflammatory treatment, and frequent milking. The low clinical recovery rate of our placebo-treated cows (4%) is not consistent with the findings of Morin et al. (1998) and Katholm (2003), who found that 50% of dairy cows with *E. coli* mastitis recover when given the supportive approach used herein. It is possible that cows in the latter studies were largely affected by mild acute *E. coli* mastitis.

In the present study, most of the cows that were affected with *E. coli* mastitis had  $\geq 4$ th parity (48%) (Fig. 1). Moreover, the majority of cases occurred on farms that used organic bedding material such as dried manure solids (52%) or sawdust (39%). These observations are in agreement with the results of other researchers (Hogan et al., 2003; Ward et al., 2002). Since it is very difficult to treat cattle with *E. coli* mastitis, it is notable that dairy farms seek to

prevent its occurrence by instituting proper environmental sanitation. Vaccination of cows with the *E. coli* J5 mastitis vaccine is also recommended (Hogan et al. 1992).

### Conclusion

Our results suggest that supportive treatment alone does not effectively decrease the mortality rate of dairy cows with *E. coli* mastitis. Thus, cows with this disease should be treated with an effective antibiotic such as ceftiofur hydrochloride, along with intensive fluid therapy.

### Ethical approval

This study was approved by the Ethics and Research Committee of the University of Tabriz (certificate no: 012/2016).

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### Conflicts of Interest

The authors declare no conflicts of interest statement.

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