Comparison of Two Different Protocols for the Treatment of Acute *Escherichia coli* Mastitis in Dairy Cattle

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Abstract: *E-coli* mastitis is one of the most frequent causes of environmental mastitis in the dairy cattle worldwide. The purpose of this field study was to compare the efficacy of ceftiofur (HCL) in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows affected with naturally occurring acute form of *E. coli* mastitis. From January 2014 to December 2016 a total number of 100 cows naturally affected by acute *E-coli* mastitis randomly were allocated into two groups. A milk sample from the affected quarter was collected for bacteriological tests on the first day of treatment. In group A (control), fifty cows received ceftiofur (HCL) 1mg/5kg/BW, flunixin meglumine 2.2mg/kg, calcium borogluconate 40%, 250ml and hypertonic saline (Nacl 7.2 %,) 5ml/kg. In group B (treatment, n=50), cows received the same drugs mentioned for group A, except ceftiofur (HCL) which replaced by placebo. In the group A, 41cows (82%) and in the group B, 2 cows (4%) were survived respectively. The rates of quarter health recovery in the groups A and B were 31.7% and 0% respectively. The differences between two groups were significant (P≤0.01).

In conclusion our results indicated that treatment of cows affected with naturally occurring acute form of *E. coli* mastitis without application of effective antibiotic(s) such as ceftiofur (HCL) and fluid therapy almost impossible.

Keywords: Dairy cow, *E-coli*, Mastitis, ceftiofur.

INTRODUCTION

Escherichia coli mastitis is one of the most frequent causes of environmental mastitis in the dairy cattle worldwide including Iran. It is normal inhabitant of soil; digestive tract and manure. *E. coli* multiplies in contaminated bedding and inters the mammary gland through the teat sphincter when teat ends come in contact with this bacteria. Once *E. coli* enter the quarter(s) of mammary gland, they multiply rapidly. As they are killed by the cow's immune system, *E. coli* release endotoxins into the cow's circulatory system. Septicemia occur in32% to 75% of cows with naturally occurring coliform mastitis and many of the clinical signs such as high fever, watery and serum-like of milk, inflammation of the mammary gland, lack of appetite, diarrhea, recumbency and dehydration, all caused by *E. coli* endotoxins [1]. Indeed, endotoxins are the outer membrane of many gram-negative bacteria includes a complex lipopolysaccharide (LPS) whose lipid portion acts as an endotoxin and in some bacteria such as *E. coli* it is linked to the cell's peptidogly can by Braun's lipoprotein [2]. Untreated animals often die in a couple of days due to toxemia, dehydration and disseminated intravascular coagulation (DIC) [3].

Few studies have examined the effect or benefits of treatment on the course of gram-negative mastitis. It is believed that for treatment of endotoxin-induced shock models in other disease such as calf scours, administration of antibiotics, anti-inflammatory agents and fluids are helpful and important [4].

Some of researcher infected cow's mammary glands with *E. coli* and induced experimentally *Escherichia coli* mastitis, however these cows recovered their health without using the antibiotics [5-7]. Also in some studies, lipopolysaccharides (LPSs) experimentally were used to reproduce the clinical signs of *E. coli* mastitis in milking cows, but all of affected cows overcame disease without mortality [8,9]. In a study carried out on Japan, recumbency associated with decreased antithrombin activity and platelet counts, increased HCT and NEFA concentrations, were the main signs of death in the cows affected by acute form of *E. coli mastitis* [10].

In another study carried out by Persson enrofloxacin-treated cows had lower SCC compared with placebo-treated cows after being treated for mastitis, however this treatment did not result in a higher probability of survival compared with placebo [2]. It has been shown that the intramammary application of antibiotics which were effective *in vitro*, does not improve the curerate and does not affect the elimination of bacteria in the mammary gland in the cases of spontaneous and induced coliform mastitis [11,12]. It has been shown that both moderate/mild and

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acute coli-mastitis may be accompanied by bacteremia [13, 14].

However, parenteral antimicrobial treatment seems reasonable in the postpartum period, associated with immunosuppression that occurs physiologically, especially in cows with systemic symptoms [14]. The aim of this field study was to compare the efficacy of ceftiofur sodium in conjunction with supportive measures versus supportive measures without antibiotic for the treatment of dairy cows affected with naturally occurring acute form of *E. coli* mastitis.

MATERIALS AND METHODS

This study was carried out in 36 dairy herds located in the suburb of Tabriz, Iran (38° 07' N and 46º 29' E) from January 2014 to December 2016.Temperature during the experiment ranged from -10 to 38℃ with annual rainfall in this region ranges from 226 to 250 mm.

The Holstein-Friesian cows naturally affected by acute clinical form of *E. coli* mastitis from dairy herds were included in this study. All cows had been kept in open shed barns, fed by corn silage, hay, soybean and complementary minerals (TMR) and were milked 3times in a day with mean production of 30kg milk/day.

A total number of 364 Holstein cows naturally affected by acute clinical mastitis were examined at the onset of disease and milk samples were collected from the affected quarter(s) according to the NMC guidelines and standards [15] into sterilized culture tubes and were submitted for bacterial culture before initial treatment to the Tabriz University Microbiology Lab. Among the 364 cow with clinical mastitis, 100cows after laboratory confirmation of acute form of *E. coli* mastitis, were selected and remainders (264 cases) with other miscellaneous causes of clinical mastitis (such as streptococcus agalactiae, staphylococcus aureus, Klebsiella pneumoniae and etc.) were ruled out from the experiment. Selected cows were randomly divided into two groups according to the ear tags (odd or even numbers): Group A (Treatment), n=50 or Group B (Control), n=50. Affected cows had the clinical signs such as high fever (≥40°C), watery and yellowish color of milk, inflammation and redness of the mammary gland, diarrhea, recumbency and dehydration. Among the different clinical signs, recumbency (Figure **1**) and serum-like of milk (Figure **2**) were the most common features in the cows with *E. coli* mastitis.

History of animals including the parity, date of mastitis, diet formulation, bed type and date of recent parturition or pregnancy were recorded carefully in special forms.

Cows in group A, were injected with ceftiofur (HCL) 1mg/5kg/BW in the neck region (IM) for 4 days, flunixin meglumine 2.2mg/kg(IM) for 4days, a single dose calcium borogluconate 40%, 250-500ml/cow(IV)and hypertonic saline (Nacl 7.2 %,) 5ml/kg(IV) both solutions on the first day. In group B, cows received the same medicines mentioned for group A, except ceftiofur which replaced by placebo (saline). The Water

Figure 1: A 6 year old recumbent Holstein cow with acute clinical form of *E. coli* mastitis.

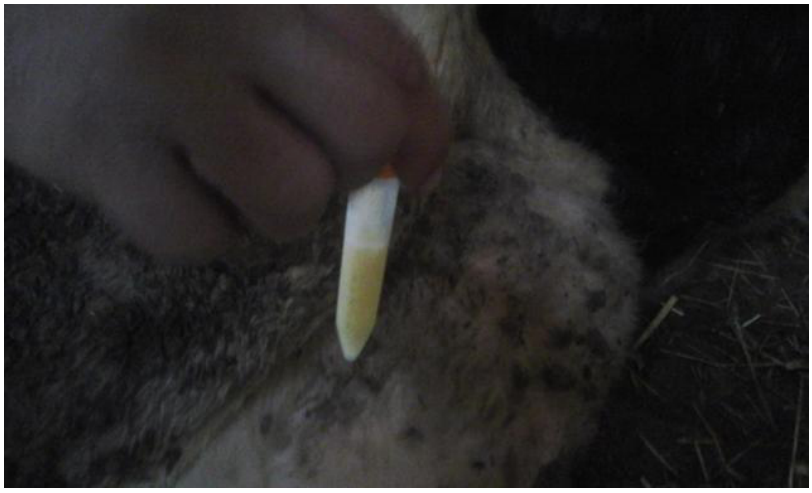

Figure 2: Watery and yellowish color of milk sample obtained from a cow quarter affected by acute form of *E. coli* mastitis.

intake intreated cows of two groups were ad libitum. Due to reducing toxic effects of *E. coli* mastitis, affected quarter(s) in all cows frequently were milked out every 2 hours. All cows in two groups were examined daily for body temperature, heart rate, appetite and probable changes in milk color of affected quarter(s). On the 5th day of treatment all survived animals subjected to the clinical examination and milk sampling from the affected quarter(s). Normalization of color and consistency of affected quarter's milk and negative *E. coli* culture result considered as quarter recovery.

RESULTS

Of 100 cows affected by *E. coli* mastitis in present study 3 animals (3%) were heifer and the remainder were multiparous cows with 2nd(12%), 3rd(37%) and ≥4th (48%) parity (Figure **3**).

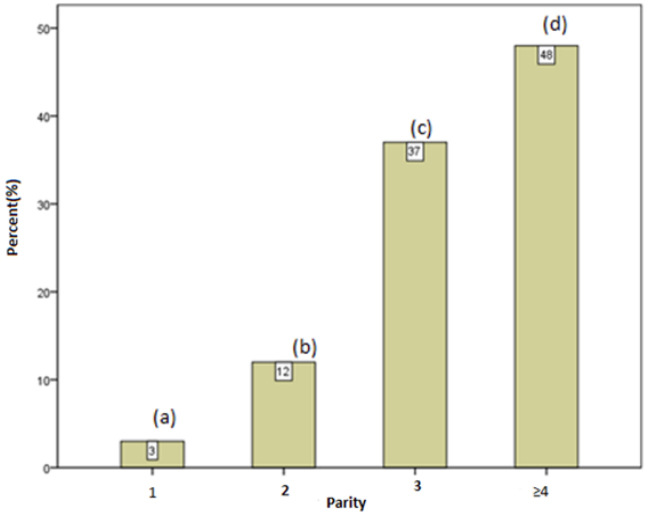

Figure 3: The rates of *E. coli* mastitis in the cows with different parity (Different letters indicate statistically significant difference between groups) (P≤0.01).

The type of cow's bed was very important and the maximum *E. coli* mastitis rate was seen in the cows with dried manure solids (DMS) (52%), following in the cows with saw dust beds (39%). The minimum rate of *E. coli* mastitis was detected in the cows with sand beds (free-stall barns) (9%) (Figure **4**).

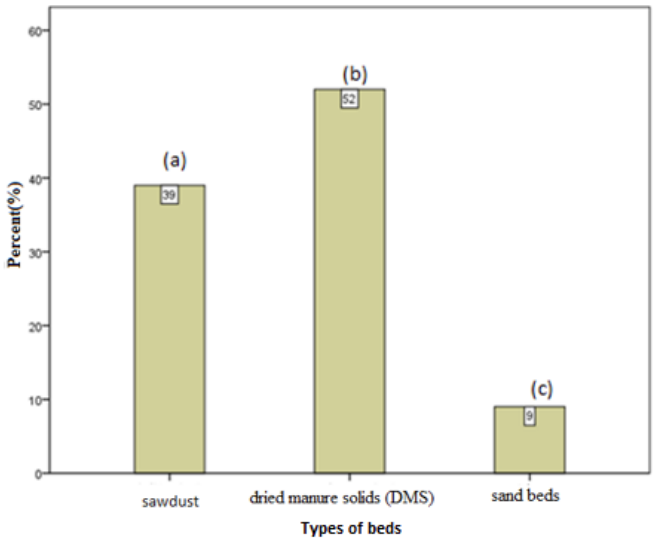

Figure 4: The rates of *E. coli* mastitis in the cows with different types of beds (Different letters indicate statistically significant difference between groups) (P≤0.01).

In the group A, 41 out of 50 cows (82%) and in the group B, only 2 cows (4%) were survived and the remainder of cows in two groups were slaughtered due to the severity of disease and failure of the treatment procedure to improve their condition (Figure **5**).

The Slaughter of cows were considered as natural death following *E. coli* mastitis. The recovery rate of quarters with *E. coli* mastitis in the groups A and B were 31.7% and 0% respectively (Table **1**).

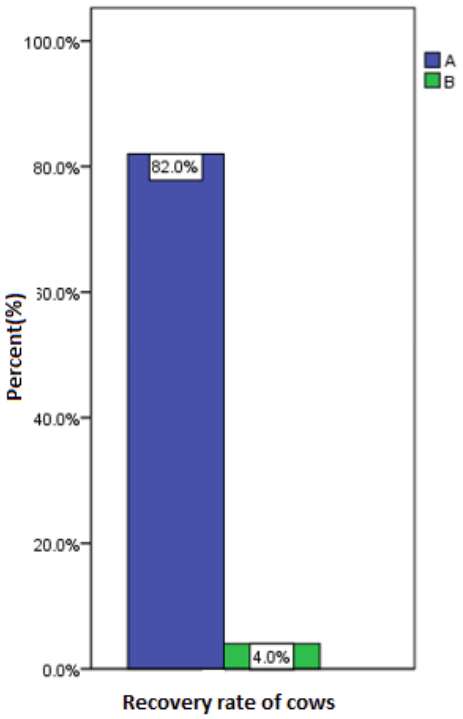

Figure 5: Recovery rate of cows in two groups A and B.

Table 1: Recovery Rate of Quarters Following Treatment of Cows with Ceftiofur (HCL) in Two Groups A and B

Group	Number of cows survived	Recovery Rate of quarters
A [ceftiofur (HCL)]	41	13(31.7%)
B (without antibiotic)	2	0(0%)

Chi-square test were used (by using SPSS software version 22, Inc., Chicago, IL, USA) for analysis of data in the present study. Statistical differences were considered significant when P<0.05 and trends are discussed when P<0.01.

The differences between two groups in the cases of cow's survival rate and quarter's recovery rate were significant (P≤0.01). Also the differences among groups in the cases of cow's parity and bed's type were significant (P≤0.01).

DISCUSSION

Escherichia coli is a major economic cause of mastitis on the modern dairy farms. *E. coli* mastitis in cows can range from being a subclinical mastitis to a highly lethal disease with prominent clinical signs. It has been approved that both moderate and acute *E. coli* mastitis may be accompanied by bacteremia and septicemia [13, 14]. Therefore, it seems reasonable that parenteral antimicrobial therapy is used concurrently with fluid therapy for the treatment of cows with acute *E. coli* mastitis. Especially it is very important subject that during the postpartum period due to physiological immunosuppression needs for parenteral antimicrobial therapy are more being felt in dairy cows. Evidence for the efficacy of the local antimicrobial treatments in *E. coli* mastitis is limited [12, 16]. Persson *et al*. [2] and Bargi *et al*. [17] used enrofloxacin for the treatment of acute clinical mastitis caused by Escherichia coli but this treatment did not result in a higher probability of survival compared with placebo. Our results indicated that parenterally using an effective antibacterial agent such as ceftiofur hydrochloride is very effective to reduce mortality rate of affected cows (82% vs 4%) (Figure **5**). This finding in agreement with the Erskine's research which showed that in the cases of severe clinical mastitis caused by coliform organisms, ceftiofur therapy could reduce the proportion of cases that lead to death [18]. Ceftiofur is a third-generation of cephalosporins and effective against a wide range of Gram-positive and Gram-negative bacterial pathogens that cause mastitis [19]. Also our results are in agreement with the Suojala's *et al*. [16] research that recommended fluoroquinolones, or third- or fourth-generation cephalosporins for the treatment of acute *E. coli* mastitis due to bacteremia and septicemia following growth of bacteria in the mammary gland But our finding in contrast with the Morin's *et al*. [20] and Katholm's [21] researches which suggested a non-antimicrobial approach (anti-inflammatory treatment, frequent milking and fluid therapy) for the treatment of *E. coli* mastitis in the affected cows. They treated 50% of affected cows by supportive therapy alone, without using antibiotics, but in our study in the control group 96% of cows affected by *E. coli* mastitis were slaughtered due to not using antibiotics. Probably the cows in the Morin's and Katholm's studies had not been affected by acute form of *E. coli* mastitis and most of them affected by mild form of this type of mastitis.

As mentioned above most of affected cows in present study were belonged to the old cow's groups [parity ≥4th (48%)] (Figure **3**) and to the farms that there were used organic beds such as dried manure solids (DMS) (52%) or saw dust beds (39%). These results in agreements with the results obtained by other researchers such as Hogan and Ward [12, 22]. Treatment of cows with *E. coli* mastitis is very difficult procedure, so for prevention of disease in the high risk

farms and regions proper environmental sanitation and vaccination of cows by *Escherichia coli J5* mastitis vaccine recommended [23].

CONCLUSION

In conclusion our results indicated that, in the cases of acute *E. coli* mastitis vigorous antibiotic therapy such as ceftiofur hydrochloride and supportive treatment necessary for saving of the affected cows and supportive treatment alone not effective for saving the affected cows.

SOURCES AND MANUFACTURES

a. Ceftiofur [CEMAY, 100ml injectable vial, 1 ml contains: 50 mg Ceftiofur (HCL), produced by Maymó laboratories, Spain.

b. Sodium Chloride 7.2 %(Salinjet, 1000ml injectable solution, 1 ml contains 7.2 mg Nacl produced by Zoopha Parnian Pars, Iran.

c. Flunixin Meglumine (Flunex 5%, 50 ml injectable vial, each ml contains 50 mg flunixin meglumine produced by Razak Labs. Co. Iran)

d. Calcium borogluconate (Calcibor 40%, manufactured by Nasr Company, Iran) 250ml.

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