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ORIGINAL ARTICLE

Effect of Intravenous Infusion of Hypertonic Saline on Treatment of the Cows with Acute *E. Coli* Mastitis

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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 study was to evaluate the efficacy of hypertonic saline infusion on the treatment of cows with acute E-coli mastitis. 100 recumbent cows with acute clinical mastitis, detected as E-coli mastitis by the laboratory of Tabriz veterinary faculty, from January 2013 to January 2015. The cows were randomly allocated into two treatment regimens. In group A (control, n=50), cows received enrofloxacin 2.5mg/kg, flunixinmeglumine 2.2mg/kg,calciumborogluconate 40%, 250ml and isotonic saline 5ml/kg(Nacl 0.9%). In group B (treatment, n=50), isotonic saline replaced by hypertonic saline 5ml/kg (Salinjet, Nacl7.2 %,) and the same drugs were administered to the cows. All cows in two groups had access to water ad libitum. 26 out of 50 cows in group A (52%), died due to septicemia and 14 out of 24 recovered cows (58%) lost one of her quarters due to acute mastitis. Mortality rate in group B was 9 cows (18%) and the number of blinded quarter were 11cases (22%). The differences between two groups were statistically significant (P≤ 0.05). In conclusion infusion of hypertonic saline has the main role in the treatment of cows with E-coli mastitis.
Key words: Cow, E-coli, Hypertonic, Saline, Mastitis,

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INTRODUCTION

Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae and *Serratiam arcesans* are four common coliform bacteria that cause mastitis. Coliform bacteria are normal inhabitants of soil; digestive tract and manure. They accumulate and multiply in contaminated bedding. Coliforms invade the udder through the teat sphincter when teat ends come in contact with coliform bacteria. Once coliform bacteria enter the mammary gland, they multiply rapidly. As they are destroyed by the cow's immune system, coliforms release endotoxins into the cow's body. These endotoxins cause many of the clinical signs such as high fever, abnormal milk, inflammation of the udder (usually just one quarter), lack of appetite, dehydration, diarrhea and recumbency. Death can result from endotoxin released by the bacteria overwhelming the cow. Milk color is serum-like and consists of large white flakes [1].Often severe cases occur in older high producing cows early in their lactation. E-coli is a major economic cause of mastitis on the modern dairy farms. Although few studies have examined the effect or benefits of treatment on the course of gram-negative mastitis, extrapolation from endotoxin-induced shock models in other species suggests that effective treatment of gram-negative mastitis would include administration of antibiotics, anti-inflamatory agents, and fluids as well as frequent emptying of the affected gland [2]. However many of clinicians rarely treat the adult cattle with IV fluids because the volume of fluid required, 40-60 L/d, [3] and the time, difficulty, and expense of treatment. Furthermore, to our knowledge, no studies have examined the efficacy of fluid therapy in the treatment of gram-negative disease in mature cattle. Small volumes of hypertonic solutions can be transported easily and administered rapidly, which are distinct advantages in ambulatory practice [4]. Hypertonic saline solutions have shown promise in human beings, laboratory animals, and livestock neonates [5, 6]. Studies demonstrated that administration of hypertonic NaCL solutions was followed by rapid expansion of circulating plasma volume, a positive endpoint in the treatment of shock. [7,8]. The purpose of present study was to determine the effect of IV administration of hypertonic saline on the treatment process of dairy cows with acute E- coli mastitis.

MATERIALS AND MTHODS

A total number of 100 cows with acute clinical mastitis detected as E-coli mastitis by the laboratory of Tabrizveterinary faculty, from January 2013 to January 2015. The clinical signs of those cows were including of anorexia, swollen and painful quarters, acute mastitis, recumbence, weakness, high fever, diarrhea and dehydration. For confirmation of E-coli mastitis milk samples from affected quarters were inoculated on MacConkey Agar and incubated aerobically at 37^{oC} for 24 hours. The plates were observed for the growth of *E. coli*. A single, isolated colony was picked and subcultured again on MacConkey agar for purification of the isolate. Simultaneously another single colony with similar characters was picked for the preparation of smear and stained with Gram's stain for the examination of staining and morphological characters of the isolate using bright field microscope. The cultural characteristics of the isolates were confirmed by inoculating the pure colonies on Blood Agar, Nutrient Agar, Nutrient Broth and Violet Red Bile Agar. Biochemical tests were performed to confirm the *E. coli* using catalase test, Simmon's Citrate Agar, sugar fermentation on Triple Sugar Iron Agar, Gelatin liquefaction, Indole Production, Nitrate reduction, Urease production, Vogesproskaur, Methyl red and Presumptive test.

Then, affected cows were randomly allocated into two treatment regimens: in group A (control, n=50), cows received enrofloxacin (Enrovet 10%, manufactured by Abureihan Company ,Iran) 2.5mg/kg, flunixinmeglumine (Vetafluxin, manufactured by Abureihan Company ,Iran) 2.2mg/kg, calcium borogluconate (Calcibor 40%, manufactured by Nasr company, Iran) 250ml/cow and isotonic saline 5ml/kg (Nacl 0.9%, manufactured by Gazi-Tabatabaee Company, Iran). In group B (treatment, n=50), cows received hypertonic saline 5ml/kg (Salinjet, Nacl 7.2%, manufactured by Zofa-Parnian pars Company, Iran) plus the same drugs mentioned for group A, except isotonic saline. All cows in two groups were milked out every 3 hours and had access to water ad libitum. The duration of treatment was 3days for all of cows but fluid therapy were carried out only on the first day of treatment.

RESULTS

26 out of 50 cows in group A (52%), died due to septicemia and 14 out of 24 recovered cows (58%) lost one of her quarters due to acute mastitis. Mortality rate in group B was 9 cows (18%) and the number of blinded quarter were 11cases (22%) (Table1). The results compared by Chi-square using SPSS software, version 16 (GraphPad Software, California, USA). Differences between two groups were statistically significant (P≤ 0.05).

Table 1.The rate of mortality and blinded quarters in two groups (A&B) after treatment.

Group	Mortality rate	Recovery rate	Number of cows with blinded quarters	Total number of cows
A(Isotonic saline)	26cows (52%)	24 cows (48%)	14 (58%)	50
B(Hypertonic saline)	9 cows (18%)	41 cows (%82)	11 (26%)	50

DISCUSSION

Bovine mastitis is one of the most complex and costly disease in the dairy industry due to its high prevalence and economic losses (9). Field studies in herds with low somatic cell counts from Pennsylvania, Ohio, and California have reported average herd incidence of clinical mastitis to be 45 to 50 cases/100 cows annually (10, 11 and 12). Coliform bacteria are responsible for a great number of acute clinical mastitis cases in dairy cows Although decreased milk production associated with subclinical mastitis is the largest cost associated with intramammary infections (13) clinical mastitis is probably of greater economic consequence on many well-managed dairies (14).Our results indicated that in the case of acute E. coli mastitis rehydration very important and essential step in the treatment process of affected cows. Cows which received hypertonic saline solutions had the more chances to survive than the cows received isotonic saline. Also the rate of blinded quarters in the group B (hypertonic saline) was lesser than the group A (isotonic saline). This result suggests that infusion of hypertonic saline should increase the blood circulation in the udder and improves the defense mechanisms of the affected quarters and help to healing process. Administration of hypertonic saline solution for other livestock diseases has already been reported. These fluids are believed to draw water from interstitial or intracellular fluid compartments, increasing blood volume, and consequently, improving the perfusion of peripheral tissues (15).On the basis of results obtained by Wenz et al (2001) there should be a high index of suspicion of bacteremia in cows with severe systemic disease signs; parenteral antimicrobial therapy may be indicated in such cases of acute coliform mastitis. However, according to our results antibiotherapy without fluid therapy is not a fully successful treatment. Also in present study, there was a distinct seasonal pattern of E-coli mastitis associated with low temperatures and heavy rainfall during the autumn and winter seasons. Mastitis caused by *Escherichia coli* is common in high-producing cows with a low milk somatic cell count. In these herds, control and eradication efforts have decreased the prevalence of contagious- udder pathogens but

little attention have paid for environmental mastitis. General cleanliness, correct stocking densities and a comfortable clean dry bed all have a beneficial effect in preventing E-coli and other forms of environmental mastitis. Good drainage and ventilation reduce humidity and lead to dry conditions that have a major effect on the coliform bacteria. The quality and management of bedding is of great importance. Straw that has become wet in storage or was baled at high moisture content has reduced absorbency and can predispose to mastitis. Sand is a good inert bedding material, which minimizes all bacterial growth. Teat hygiene and preparation must be fastidious. Dry teats are essential for milking; a dry wipe should in most cases be all that is required. It has been a benefit in some herds to pre-disinfect and wipe the teats before milking.One of the simplest recommendations is to keep cows standing in a clean area for 30 minutes after milking to allow for adequate teat end closure, so creating a physical barrier to contamination. On farms with continuing problems, vaccination can be a useful tool for prevention; and should be noted in herd health plan. Mammary resistance to coliform (and all gram-negative) intramammary infections can also be enhanced by use of J5 (R mutant) *E. coli* antigen vaccines. They have proven safe and efficacious in reducing the incidence of clinical coliform mastitis in dairy herds [17, 18 and 19].

In conclusion infusion of hypertonic saline and fluid therapy has the main role in the treatment of cows with acute E-coli mastitis.

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