Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Field Study on Cefquinome in Treatment of Clinical Bovine Mastitis

Nashwa A. Omar[1*], Mona F. Eltalawy[2], Amal F. El-Zoghby[1] and Amr A. El-Samahy[3]

[1]*Department of Pharmacology, Animal Health Research Institute (AHRI), Tanta Branch, Agricultural Research Center (ARC), Egypt.*

[2]*Department of Food Hygiene, Animal Health Research Institute (AHRI), Tanta Branch, Agricultural Research Center (ARC), Egypt.*

[3]*Department of Biochemistry and Feed Deficiency, Animal Health Research Institute (AHRI), Tanta Branch, Agricultural Research Center (ARC), Egypt.*

Abstract

THE CURRENT study aims to identify the main pathogens responsible for mastitis, and to investigate the effect of extended treatment of both cefquinome and cefquinome-diphenhydramine combination in treatment of mastitis in cows, with evaluation of its residues in milk after treatment. Besides, serum biochemical analysis including AST, ALT, creatinine, urea, IgE, histamine, in addition to antioxidant status (GSH and MDA) were also assessed. The study used 20 native mixed breed dairy cows which were categorized into four groups made up of five cows in each group; (G1): clinically healthy and negative to California test (CMT) as a negative control group, (G2): mastitic +ve CMT as a positive control group, (G3): mastitic +ve CMT and given therapeutic dose of Cefquinome alone, (G4): mastitic +ve CMT and treated with therapeutic doses of Cefquinome and diphenhydramine. The obtained results revealed that coagulase negative *Staphylococcus aureus*, coagulase positive *Staphylococcus aureus*, *Streptococcus agalactia* and *E. coli* were isolated from clinically mastitic cases. In residue depletion study, Cefquinome remained detectable in treated cows` milk until the 10th day of treatment (0.006±0.002µg/ml), although it was below the Maximum Residual Limit (20 µg/kg). In pharmacodynamic study, cefquinome–diphenhydramine treatment resulted in significant attenuation of mastitis-associated increases in serum MDA, IgE, and histamine concentrations, while maintaining normal hepatic function parameters, as evidenced by unaltered serum AST and ALT activities. It could be concluded that cefquinome–diphenhydramine treatment exhibits superior therapeutic efficacy over cefquinome monotherapy in bovine mastitis, as demonstrated by enhanced modulation of inflammatory markers and improved antioxidant status.

Keywords: Mastitis**,** Antibiotic residues, Cefquinome, Diphenhydramine.

Introduction

Mastitis is one of the most prevalent, expensive, and seriously affecting diseases in dairy cattle [1]. Mastitis prevalence in dairy cattle is estimated from 5 % to 36 % in herds during single check and up to 68% over a year [2]. It affects cattle over the world, and most measurements have shown that it reduces productivity by 30% per affected quarter and milk production by 15% per cow per lactation [3]. Costs associated with mastitis include lower milk output, discarding of milk due to antibiotic residues, veterinary services, culling of chronically diseased cows, and occasional death [4]. This is especially important to farmers in developing nations [5]. Moreover, mastitis also carries a significant risk of zoonotic transmission due to releasing the bacteria and their toxins into milk [6]. Bovine mastitis mainly cognate by microorganisms which typically including both bacteria, yeasts, viruses and *Mycoplasma* [7].

Over the past few decades, numerous farms across various countries have effectively reduced the prevalence of contagious mastitis by implementing five strategies: improving milking techniques and milking machines, treating cow blankets, cleaning teats after milking, treating clinical mastitis, and killing cows with a persistent infection [8]. The average bulk milk somatic cell count (BMSCC) has dropped significantly across most European countries due to the fall in infectious bacteria including *Staphylococcus aureus* and *Streptococcus agalactiae*. Regardless, even at low BMSCC levels, *Staph.*

*Corresponding authors: Nashwa A. Omar, E-mail: dr.nashwa_omar@hhotmail.com Tel.: 010 00770898
(Received 11 November 2024, accepted 26 January 2025)
DOI: 10.21608/EJVS.2025.335220.2488
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aureus is still a major mastitis-causing bacterium on many farms [9].

A common practice to extend the period of treatment and maximize the likelihood of a cure; nevertheless, there has been insufficient and inconclusive research on the efficacy of prolonged antibiotic treatment for clinical *S. aureus* mastitis, with the results indicating either a numerical improvement or a substantially greater rate of cure for instances with β-lactamase negative *S. aureus* [8]. Therefore, additional studies examining the outcomes of prolonged therapy for clinical *S. aureus* mastitis are required.

Physicochemical changes in milk, an increase in somatic cell count (SCC), bacterial pathogens, changes in mammary tissue, and the ability to notice effects on milk quality and quantity are all characteristics of intramammary infections (IMIs), the specifics of which vary by disease type [10, 11].

One example of a fourth-generation cephalosporin antibiotic is cefquinome, also known as cefquinome sulfate. The majority of the microorganisms that cause cow mastitis are susceptible to cefquinome's antimicrobial properties [12]. Its strong antibacterial characteristics led to its veterinary approval for the treatment of numerous diseases in cattle, pigs, sows, and cows in European countries [13]. Diphenhydramine is an antihistaminic substance. It acts like other antihistaminic through inhibition of histamine at H1 receptors [14].

Antibiotic residuals in milk not only affect the product's quality but also pose a serious health risk to consumers [15]. Customers who consume this milk run the risk of developing allergies, digestive changes, and microorganisms that are resistant to many drugs [16]. Additionally, antibiotic residues may hinder the milk's normal microbial balance and negatively impact several dairy products' production processes, including yoghurt and cheese [17].

The present study sought to identify and isolate the primary organisms responsible for mastitis, as well as to examine the effects of prolonged therapy with cefquinome (CEQ) alone and in combination with diphenhydramine on mastitic cows and the resultant residues in milk samples post-treatment. Additionally, serum biochemical analysis encompassing Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), creatinine, urea, IgE, histamine, and evaluation of antioxidant status through Reduced Glutathione (GSH) and Malondialdehyde (MDA) was conducted.

Material and Methods

Medication

Cefquinome sulphate

Cefquinome LC injectors - intramammary (each injector contains 75 mg cefquinome; Intervet International GmbH, Germany) at a single dose in affected quarter after milking each 12 hours for three successive days.

Cefquinome 2.5% injectable solution - intramuscular (1 mg/kg Cefquinome IM once daily for three successive days), [18].

Diphenhydramine HCL

Diphenhydramine; Pharma Swede Egypt, 10th of Ramadan City-B3. At a dose of 50 mg Diphenhydramine HCL / 100 kg Once IM [19].

Approval number

The study was licensed by the approval No. ZU-LACUC/2/F/401/2022.

Experimental design and treatment

The research was done in a private farm in Kafr El-Ziat, Gharbyia Governorate and Animal Health Research Institute (AHRI), Tanta Lab. and Dokki Lab. chemistry department (chemistry & pharmacology units).

A total of 100 milk samples were collected from cows and outwardly healthy cows in the farm. Every sample was by CMT [20].

Only lactating cows with clinical mastitic (CM) symptoms in one quarter were required to meet the first inclusion criterion. Cows exhibiting clinical symptoms in more than a quarter of them were excluded from the study.

Clinical examinations of the investigated dairy cows were performed [21]. Before sampling, udder of each animal was checked for any changes in clinical mastitis symptoms (symmetry, inflammation, hotness, swelling, pain sensation or any physical alterations). Visual examination of milk per quarter was done using the strip-cup test to observe any abnormalities in milk samples (viscosity, milk clots, flakes, pus, bloody and watery secretion).

Twenty dairy cows (native mixed breed) of 2-3 years old with 500-600 kg were fed twice daily after each milking, while being kept outside in pens with concrete floors and open front sheds for shelter. Half the ration was offered after each milking with free access to clean drinking water**.**

All cows were examined once daily for 10 days of treatment or till resolution of symptoms. Body temperature, heart and respiratory rates were recorded daily.

Twenty dairy cows were divided into 4 equal groups (five of each). The first group (G1) the clinically healthy -ve CMT and kept as a negative control group and was not given any treatment. The other three groups include the mastitic cows +ve CMT which suffered from signs of mastitis including swelling, heat, hardness, redness, and pain of the udder, some of it expressed systemic signs including

fever, depression, and off-food. The second group (G2) was kept as infected non-treated positive control. The third group (G3) was given appropriate doses of treatment of Cefquinome. The fourth group (G4) was treated with therapeutic doses of Cefquinome and Diphenhydramine.

Collection of milk samples

Two samples of milk were collected from each investigated animal under aseptic conditions in sterile McCarteny. The first sample was subjected to bacteriological examination before and after treatment and the second sample was centrifuged at 3000 rpm for 15 minutes, and the fat-free liquid (skimmed milk) was stored at -20°C for detection of cefquinome residues in milk samples using HPLC in the 4^{th}, 7^{th}, 10^{th} days from the beginning of drug administration. All samples were sent immediately in an ice container to the laboratory for examination.

Collection of blood samples

Jugular venous blood samples were collected at the 4^{th} and 10^{th} days of study into free-anticoagulant glass tubes that were left to clot then centrifuged at 3000 rpm for 20 minutes. The obtained sera were stored frozen at −20°C until be tested biochemically.

Antimicrobial susceptibility

The following antimicrobial discs were used *in vitro* to test the causative agents for their susceptibility: Cefquinome (CEQ10), amikacin (Ak30), gentamycin (Gen30), enrofloxacin (Enr5), ciprofloxacin (Cip5), penicillin (p 10) and amoxicillin-clavulanic (Amc30) [22, 23].

Bacteriological examination

Milk samples were examined bacteriologically to evaluate total bacterial count (T.B.C.) before and after treatment (ISO 4833-1:(2013). Besides, total staphylococci count isolation and identification of *S. aureus***,** total *streptococci* count, isolation and identification of *Strept. Agalactiae*, total Coliform count, isolation and identification of *E. coli* were carried out according to previous methods [24-28].

Detection of cefquinome residues in milk samples

AHRI's pharmacology team used High Performance Liquid Chromatography (HPLC) to quantify cefquinome, using High Performance Liquid Chromatography (HPLC) analyses (Agilent, 1200). In brief, a 1-mL milk sample was transferred to a 15-mL polypropylene centrifuge tube, and 3 mL of 10% trichloroacetic acid was added. The mixture was briefly vortexed for 1 minute, shook for 10 minutes, and centrifuged at 9,000×g for 10 minutes at 4°C. The supernatant was transferred to a glass tube, and the residue was extracted again once. The resultant supernatant was placed into a solid phase extraction (SPE) cartridge (Strata-X, 60 mg/3 mL) that had previously been activated with 3 mL of methanol and 3 mL of deionized water at a flow rate of around 1 mL min-1. Sample solutions flowed through the SPE cartridge with gravity. The SPE cartridge was then cleaned with 3 mL of water and dried under vacuum for 15 minutes. The retained medication was eluted from the cartridge with 1 mL of 30% acetonitrile. The eluate was vortexed and centrifuged at 15,000×g for 10 minutes. After filtering with a 0.22 µm nylon syringe filter, the upper layer was collected into a small volume sample vial. HPLC was then used to evaluate the sample. Chromatographic separation was achieved at 30°C on a reversed phase column C18 (250 × 4.6 mm, 5µ) using a mobile phase consisted of 130 ml of acetonitrile with 28 ml glacial acetic acid diluted to final volume of 1000 ml with ultra-pure analytical grade type 1 water for HPLC that was produced using a water purification system (Millipore). The flow rate was kept at 1 ml/min and variable wave length UV absorbance detector was carried out at 268 nm. The injection volume was 20 µl for assay level. The limit of quantification (LOQ) of the used HPLC assay was 0.15 µg/ml while limit of detection (LOD) 0.05 µg/ml [29].

Biochemical analysis

Using a commercial kit and following the manufacturer's instructions, serum biomarkers were identified.

Spectrophotometric measurements of all parameters were made using standardized test-kits. Activities of serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were assessed [30]. Urea and creatinine were determined [31] using kits of Biodignostic, Cairo, Egypt.

Lipid peroxides formation was assessed as Malondialdehyde (MDA) [32] and Reduced glutathione (GSH) [33].

IgE: as described previously [34].

Histamine: using the techniques outlined [35].

Statistical analysis

The normality of distribution and homogeneity of biochemical tests variances between different treatments were tested using the Shapiro-Wilk test, and the assumption was achieved ($p > 0.05$). The data were statistically analyzed by ONE-WAY Analysis of Variance (ANOVA) using SPSS version 24. Duncan's Multiple Range Test was utilized to assess statistical differences between the groups at a significance level of 0.05 [36]. The data were expressed as mean ± standard error (SE).

After collecting data of bacteriological examination, we used SPSS version 25 to analyze it. As the analysis of variance did not produce any significance between groups, we had to perform median test to find out if there was difference between them or not. Median (IQR), declared the

value of median across groups and the interquartile range of measurements to see how spread out the data around the median [37].

Results

The results of the clinical and subclinical mastitis were estimated by CMT among the mastitic cows and outwardly healthy cows showed that out of 100 examined milk sample, 72% of samples were CMT negative. On the other hand, 13% of samples were CMT positive, so 15 cows had subclinical mastitis with 15% of total examined cows.

Table (1) declared that all isolates were high prevalence sensitive to cefquinome (100%), followed by Amikacin, gentamycin (93.3%), enerofloxacin (80%) and ciprofloxacin (70%).

There were statistically significant differences between control and treatment groups after four and seven days of treatment with cefquinome alone and cefquinome- diphenhydramine. With 95% confidence interval, all p-values were less than the level of significance ($\alpha = 0.05$). Total *staphylococci* were decreased at the 4thday of treatment and still present till 7th day in treated groups with cefquinome alone and cefquinome- diphenhydramine, as shown in table (2).

Table (3) shows that the prevalence rates of *E. coli*, *Staph. aureus* (CNS, CPS), and *Streptococcus agalactiae*. CNS and CPS in G3 before treatment were 66.7% and 33.3% respectively. While incidence rates of CNS only in G4 were 66.7% between clinical cases.

Cefquinome residues:

The mean milk concentration of CEQ 24 hrs. after last IM dose was 0.193 ±0.04 µg/ml and then declined to 0.019±0.004 µg/ml at 7th day from beginning of treatment, cefquinome was still detectable at 10th day from beginning of treatment (0.006±0.002µg/ml) which was for below the Maximum Residual Limit (MRL) (20 µg/kg).

Biochemical analysis:

Table (5) revealed that G2 (infected non treated group) showed a significant increase in serum AST and ALT activities compared with G1 (negative control group) along the whole experimental period. Whereas, results of serum AST and ALT activities in G3 (cefquinome-treated group) and G4 (cefquinome-diphenhydramine-treated group) showed non-significant changes compared with G1 all over the experimental period. Regarding serum levels of urea and creatinine; there was a significant increase in G2 compared with G1 throughout the experiment. Conversely, G3 and G4 expressed a significant decrease in serum levels of urea and creatinine compared with G2 across the entire experimental period. Our finding clarified that G2 exhibited a significant increase in serum levels of MDA, IgE and histamine, while demonstrated a significant decrease in GSH serum level compared with G1 during the experimental period. On the other hand, GSH levels significantly increased in G3 and G4 compared with G2 across the experimental period. Concerning serum levels of MDA, IgE and Histamine; G3 and G4 showed a significant decrease compared with G2 all-over the experimental period.

Discussion

The antimicrobial investigation of diseases is crucial as it provides a protocol for antibiotic therapy, reduces antibiotic resistance, and lowers the risk to the public health [38].

The results of clinical and subclinical mastitis estimated by CMT among the mastitic cows and outwardly healthy cows showed that out of 100 examined milk sample, 72% of samples were CMT negative. On the other hand, 13% of samples were CMT positive, nearly similar result as shown in a previous study [3], which demonstrated an incidence rate of 10.3% for mastitis at quarter level. Also, in the present study 15 animals (15%) had subclinical mastitis which agrees with a previous report [39] who determined the incidence of subclinical mastitis to be 16.3%. The difference in the prevalence of mastitis between this study and others may be attributed to differences in environmental and management protocols, such as soil, floor and use of straw or hay bedding increased the occurrence of mastitis.

Antibiotic susceptibility testing was examined for *Staphylococcal* species, *Streptococcal* species and *E. coli* which were isolated from the tested milk samples and showed that all isolates were resistant to penicillin and co-amoxiclav. Likely, Tavakoli and Pourtaghi [40] recorded high resistance to penicillin and ampicillin. Interestingly, all isolates showed high sensitivity to cefquinome (100%), followed by amikacin and gentamycin (93.3%), enerofloxacin (80%) and ciprofloxacin (70%) (Table 1), which agreed with a previous report [41]. Cefquinome is the best choice antibiotic in this study for treatment of mastitis caused by *Streptococcal* and *E. coli* species. Adding of diphenhydramine in treatment of mastitis reduced the count of *S. aureus* when compared to the group which treated by cefquinome only (Table 2). The main cause of treatment failure is due to the heterogeneity of different bacteria' responses to various antibiotics and the random, use of these medications. To avoid high expenditures for owners, testing with agar gel diffusion test for antibiotic medications must be used to determine the best course of treatment [42].

As shown in table (2) there was a statistically significance differences between control and treatment groups after four and seven days of treatment with cefquinome only and cefquinome & diphenhydramine. The present study revealed that the

negative control group had a total bacterial count less than 100 cfu/ml while it was free from any of *staphylococci*, *streptococci* and *coliform.* The total colony count is considered an indicator in the dairy farm of the general hygienic condition and the health status of the udder [43]. The primary sources of milk contamination are infected udders and/or teats, animal skin, feces on the udder, contaminated milking and storage equipment, and water used for cleaning [44]. Therefore, it is advised to count particular groupings of bacteria such *Staph. aureus* and *coliform i*n addition to employing aerobic plate count to assess the hygienic quality of milk [45].

Total *staphylococci* were decreased on the 4th day of treatment and still present till 7th day in treated groups with cefquinome alone and cefquinome-diphenhydramine, while total *streptococci* count was decreased on the 4th day of treatment and disappeared on the 7th day of treatment. On the other hand, total *coliform* counts were diminished on the 4th day of treatment. This could be attributed to the fact that cefquinome is an appropriate antibiotic for treatment of mastitis caused by *staphylococci* spp. Likelt, cefquinome is regarded to be effective in the treatment of *streptococci* and *coliform* spp [8].

Table (3) shows the prevalence rates of *E. coli, S. aureus* (CNS, CPS), and *Streptococcus agalactiae*. CNS and CPS in G3 before treatment were 66.7% and 33.3%, respectively. It was found that the incidence rates of CNS among clinical cases in G4 were 66.7%. Nearly similar results were shown in a previous study Abo Zaid et al., [46], who found that the incidence rate of CNS was 60.9% in clinical cases. On the other hand, G3 and G4 contained 40% of *Streptococcus* agalactiae cases and 20% of *E. coli* cases. Researchers obtained nearly similar results, stating that the percentage of *E. coli* isolated from clinical cases was 19.6% [47]. These outcomes were also closely related to Serdal and Funda [48], who declared that the most common pathogens were *E. coli* (19.9%).This dairy herd identified *S. aureus* as the primary pathogen causing clinical mastitis. Cefquinome treatment did not eliminate this pathogen from milk samples, but it reduced the prevalence of *Strept. agalactiae* and *E. coli*. This condition results in *S. aureus* cells sporadically shedding from inflamed mammary glands into raw milk [49]. Consequently, the existence of high *S. aureus* concentrations in a dairy herd is a sign of mastitis. The surface of the udder is where *S. aureus* lives and breeds. It can get deep into the tissue and cause deep-seated foci, which may explain why the bacteria are more common [50]. In order to lower the prevalence of mastitis, strict hygienic measures for housing and bedding should be taken into consideration.

Cefquinome residues

Antimicrobial residues were recorded in milk as the owners do not follow the recommended drug withdrawal periods, they use drugs illegally or extra-labelly, or they use the incorrect dosage levels and delivery methods [51]. Boiling or pasteurization cannot remove antibiotic residues, posing a greater long-term health risk to the general population [52]. Ingesting milk containing antimicrobial medication residues beyond the maximum permitted limits can jeopardize a person's health and safety. One consequence is the evolution of bacteria that are resistant to antibiotics or the selection of bacteria that are resistant to antibiotics, which leads to infections in susceptible people and specific tissue damage, toxicity, and cancer. Furthermore, drug residues inhibit the growth of starter cultures that are essential for the production of cheese, cultured milk, and other dairy products [51]. Although boiling and pasteurizing milk destroy or remove dangerous microorganisms, their effects on drug residues are limited or unpredictable [51].As shown in Table 4, the mean milk concentration of CEQ 24 hours after the last intramuscular dose was 0.193 ± 0.04 µg/ml. On the 7th day of treatment, it decreased to 0.019±0.004 µg/ml. Even on the 10th day, cefquinome was still detectable at 0.006±0.002 µg/ml, which was significantly lower than the Maximum Residual Limit (MRL) of 20 µg/kg [53]. The MIC90 of CEQ is between 0.008 and 4.0 µg/ml when tested against different types of bovine mastitis bacteria, including *E. coli, Staphylococcus aureus, Streptococcus uberis,* and *Streptococcus agalactiae*. The present research shows that most of the bacteria that cause mastitis can remain in milk at a therapeutic concentration of CEQ for up to 96 hours after treatment with intramuscular or intra-abdominal injections. This is a lot longer than their minimum inhibitory concentration (MIC).

Biochemical analysis:

The infected but not treated group had significantly higher levels of AST and ALT in their serum throughout the whole experiment, which showed damage to liver cells. These results coincided with the previous studies [55, 56]. During the whole experiment, the AST and ALT enzyme levels did not change much between the groups that were given cefquinome or cefquinome and diphenhydramine and the negative control group. These findings are in agreement with the previous studies [57, 58]. These findings disagreed with previous reports due to the difference in species [56, 59].

In G2, G3, and G4, the levels of urea and creatinine in the blood were significantly higher than in the negative control group. This was true throughout the experiment. These findings agree with previous reports [57-59]. The infected non-treated group had significantly higher levels of MDA, IgE, and histamine but significantly lower levels of

reduced glutathione (GSH). This was in line with what other studies [10, 55, 56] had found. On the other hand, GSH serum level significantly increased in treated groups (G3 and G4) compared with the positive control group. These findings coincided with the previous study [56]. In the infected non-treated group, MDA serum levels went up significantly, which was in line with other reports. The CEQ-diphenhydramine group, on the other hand, had much lower MDA levels than the infected non-treated group [55, 56].The IgE serum level went up significantly in the infected non-treated group, as shown in Table 5. This was in line with what Chin et al. [57] found. This finding is similar to what Bernstein et al. [61] found. They said that patients who are at high risk for IgE-mediated (allergic or anaphylactic) reactions to penicillin and penicillin-like drugs can get an immediate hypersensitivity skin test. Therefore, they recommend diphenhydramine during cephalosporin administration to prevent anaphylactic shock in approximately 10% of penicillin-sensitive individuals. The level of histamine in the blood went up significantly in the infected group that wasn't treated, but it went down significantly in the cefquinome-treated group compared to the positive control group. This finding explains that histamine activates inflammatory responses [62]. Additionally, the cefquinome and diphenhydramine-treated group exhibited significantly lower serum histamine levels compared to the untreated infected group. This implies that individuals with mastitis should receive diphenhydramine to halt histamine release and lessen inflammation.

Conclusion

The predominant isolated pathogens in the current investigation were *S. aureus, Strept. agalactiae,* and *E. coli*. The most effective antibiotics for all bacterial cases were cefquinome, followed by amikacin, gentamicin, and enrofloxacin, respectively. However, a one-sided approach cannot successfully treat mastitis. Therefore, identifying the bacteria responsible for mastitis and selecting the appropriate treatment is crucial in the prevention, control, and treatment process. This study looked at antibiotic residues and found that cefquinome is safe for treating mastitic dairy cows because its concentration was well below the maximum residual limit (MRL) after some time of treatment. It can be concluded that cefquinome is a suitable antibiotic for treating streptococcal and coliform mastitis. It was also found that the combination of cefquinome and diphenhydramine is better at treating mastitis than cefquinome alone. This is because it led to more recovery rates, less inflammation, and fewer allergic reactions. It is suggested that more research be done to find the best ways to treat mastitis in dairy cows, with a focus on reducing the amount of antibiotic residues and looking into other treatments for mastitis that don't cause antibiotic resistance.

Acknowledgments

Many thanks for Prof. Dr. Mahmoud Arafa, Biochem. Dep. Animal Health Research Institute Dokki, for assisting with the planning and estimation of blood biochemistry.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Animal Health Research Institute (AHRI), ARC, Egypt.

TABLE 1. Antibiotic susceptibility test for mastitic milk sample

Antimicrobial disc	Concentration (µg/disk)	Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
CEQ	10	15	100%	0	0%	**0**	**0**%
AK	30	14	93.3%	1	6.6%	**0**	**0**%
Gen	30	14	93.3%	1	6.6%	**0**	**0**%
Enr	5	13	86.6%	2	13.3%	**0**	**0**%
Cip	5	12	80%	3	20%	**0**	**0**%
P	10	0	0%	3	20%	**12**	**80** %
Amc	30	0	0%	3	20%	**12**	**80** %

CEQ: Cefquinome AK: Amikacin Gen: Gentamicin Enr: Enrofloxacin
Cip: Ciprofloxacin P: Penicillin Amc: Amoxicillin-Clavulanic
No=number of animals

TABLE 2. Effect of CEQ alone and cefquinome-diphenhydramine on total bacterial, total staphylococci, total streptococci and total coliform count in examined mastitic milk samples.

Parameter	Cefquinome	Cefquinome and Diphenhydramine
	MED (IQR)	MED (IQR)
	PRE	
T.C.C	1.01×10^{7} (1.79×10^{6} – 4.7×10^{7})	7×10^{5} (2×10^{5} – 1.25×10^{7})
T. Staph	2.5×10^{4} (4.25×10^{3} – 6.23×10^{5})	2.2×10^{4} (2.5×10^{3} – 3.85×10^{4})
T. Strept	1.1×10^{5} (0 – 3.93×10^{5})	1.5×10^{2} (0 – 3.31×10^{4})
T. Coliform	7.8×10^{3}	6×10^{4}
	POST	
	4D	**4D**
T.C.C	3×10^{4} (1.85×10^{3} – 4.7×10^{5})	5.1×10^{3} (1.55×10^{3} – 4.8×10^{4})
T. Staph	3.35×10^{3} (2.5×10^{2} – 6.67×10^{3})	7×10^{2} (1.5×10^{2} – 1.32×10^{3})
T. Strept	1×10^{2} ($0-4.25\times10^{2}$)	-
T. Coliform	-	-
	POST	
	7D	**7D**
T.C.C	1×10^{3} (3.5×10^{2} – 5×10^{4})	1×10^{3} (7×10^{2} – 3.7×10^{3})
T. Staph	1×10^{3} (4×10^{2} – 4×10^{3})	5.5×10^{2} (7.5×10^{1} – 2×10^{3})
T. Strept	-	-
T. Coliform	-	-
	P-value (Sig.)	
T.C.C	0.006	0.006
T. Staph	0.026	0.043
T. Strept	0.05	0.05
T. Coliform	-	-

MED (IQR) is median and interquartile range (p-value ≤0.05)
Compare medians between groups before and after treatment for 4 and 7 days (Median & SE).

TABLE 3. Prevalence of several harmful bacteria discovered in milk samples from various ethnicities that were studied:

***S. aureus*%**							***Strept. agalactiae*%**				***E. coli*%**			
Pre				**Post**			**Pre**		**Post**		**Pre**		**Post**	
G3		**G4**	**G3**		**G4**		**G3**	**G4**	**G3**	**G4**	**G3**	**G4**	**G3**	**G4**
CNS	**CPS**	**CNS**	**CNS**	**CPS**	**CNS**	**CPS**								
66.7 %	**33.3%**	**66.7%**	**66.%**	**33.%**	**40%**	**40%**	**40%**	**40%**	**0%**	**0%**	**20%**	**20%**	**0%**	**0%**

G3: Infected treated with Cefquinome. G4: Infected treated with Cefquinome and Diphenhydramine.
CNS: coagulase negative *Staphylococci* CPS: coagulase positive *Staphylococci*

TABLE 4. Area under the curve corresponding to cefquinome concentration (ng/µl) in milk samples

Level	Area	Concentration (ng/µl)
1	5.300	**0.100**
2	51.000	**1.000**
3	260.000	**5.000**
4	511.000	**10.000**
5	1023.000	**20.000**
6	2246.800	**40.000**
7	4316.000	**80.000**

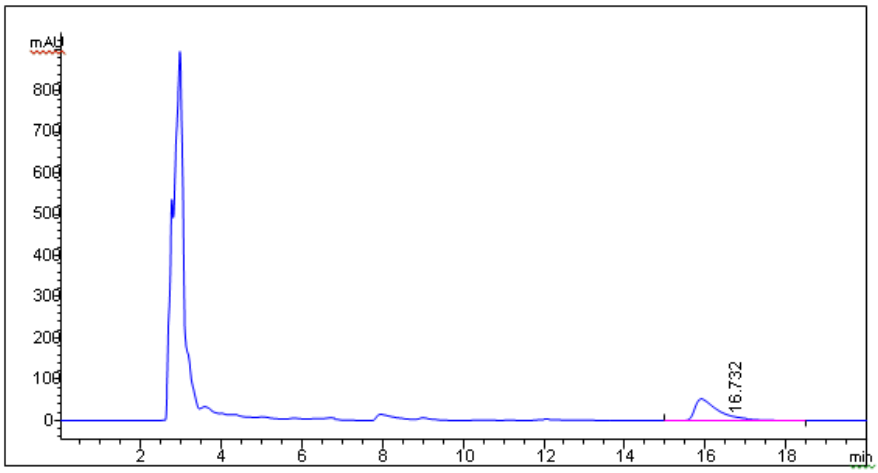

Fig. 1. Chromatogram of standard conc. 20 µg /ml Cefquinome in milk

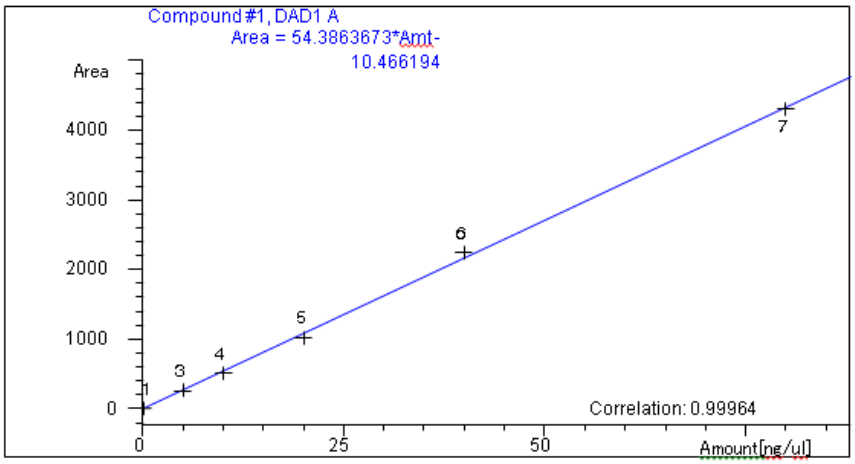

Fig. 2. Standard calibration curve of cefquinome in milk

TABLE 5. Serum biochemical parameters (mean ± SE) of mastitic cows treated with cefquinome alone and cefquinome-diphenhydramine. (n=20) at 4th and 10th days of experiment.

Parameters		4 D	10 D
Aspartate transaminase (AST) (IU/ml)	G1	47.66±1.85^{b}	50.33±1.85^{b}
	G2	56.66±2.72^{a}	68.33±1.20^{a}
	G3	49.66±1.85^{b}	52.66±2.02^{b}
	G4	48.00±1.73^{b}	50.66±2.33^{b}
Alanine transaminase (ALT) (IU/ml)	G1	25.66±0.88^{b}	28.33±1.20^{b}
	G2	35.00±2.64^{a}	41.33±1.33^{a}
	G3	27.00±0.57^{b}	29.00±1.52^{b}
	G4	26.33±0.88^{b}	28.66±1.45^{b}
Urea (mmol/L)	G1	20.00±1.15^{c}	30.00±0.57^{c}
	G2	37.33±0.89^{a}	39.00±0.58^{a}
	G3	32.01±1.14^{b}	36.35±.084^{ab}
	G4	28.67±0.88^{b}	35.01±1.16^{b}
Creatinine (mg %)	G1	0.98±0.008^{d}	0.92±0.008^{c}
	G2	1.41±0.014^{a}	1.45±0.023^{a}
	G3	1.21±0.015^{b}	1.31±0.015^{b}
	G4	1.14±0.014^{c}	1.23±0.081^{b}
Reduced glutathione (GSH) (mmol /ml)	G1	19±0.32^{a}	20±0.08^{b}
	G2	11±0.10^{d}	10±0.12^{d}
	G3	14±0.08^{c}	16±0.12^{c}
	G4	18±0.05^{b}	21±0.08^{a}

Parameters		4 D	10 D
Malondialdehyde (MDA) (mmol /ml)	G1	15±0.28 [d]	16±0.05 [c]
	G2	39±0.34 [a]	30±0.21 [a]
	G3	29±0.11 [b]	21±0.08 [b]
	G4	17±.05 [c]	14±0.05 [d]
IgE (IU/mL)	G1	103±1.15[d]	104±1.76[c]
	G2	303±4.40[a]	289±2.08[a]
	G3	220±1.73[b]	270±0.88[b]
	G4	130±0.88[c]	102±1.15[c]
Histamine (ng/mL)	G1	2.01±0.04 [c]	2.03±0.03 [c]
	G2	9.74±0.04 [a]	9.58±0.02 [a]
	G3	4.33±0.18 [b]	3.16±0.02 [b]
	G4	2.01±0.03 [c]	1.43±0.03 [d]

Results are represented as mean ± standard error.
For each parameter means within same column carrying different letters are significantly different at $P \leq 0.05$
G1: Control
G2: Infected non-treated
G3: Infected treated with Cefquinome
G4: Infected treated with Cefquinome and Diphenhydramine.

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دراسة ميدانية حول استخدام السيفكوينوم في علاج التهاب الضرع في الابقار

نشوى عبدالعزيز عمر[1]، منى التلاوي[2]، أمل فتحي الزغبي[1] وعمرو السماحي[3]

[1] قسم الفارماكولوجيا، معهد بحوث الصحة الحيوانية، فرع طنطا، مركز البحوث الزراعية ، الجيزة، مصر.
[2] قسم صحة الاغذية، معهد بحوث الصحة الحيوانية، فرع طنطا، مركز البحوث الزراعية ، الجيزة، مصر.
[3] قسم الكيمياء الحيوية والنقص الغذائي، معهد بحوث الصحة الحيوانية ، فرع طنطا، مركز البحوث الزراعية، الجيزة، مصر.

الملخص

هدفت الدراسة الحالية إلى تحديد المسببات الممرضة الرئيسية المسؤولة عن التهاب الضرع، ودراسة تأثير العلاج الممتد بكل من السيفكوينوم ومزيج السيفكوينوم-ديفينهيدرامين في علاج التهاب الضرع في الأبقار، مع تقييم متبقياته في الحليب بعد العلاج. بالإضافة إلى ذلك، تم تقييم التحليل البيوكيميائي للمصل بما في ذلك AST, ALT ,الكرياتينين ,اليوريا IgE,والهيستامين، بالإضافة إلى حالة مضادات الأكسدة (GSH, MDA). استُخْدِمَ في هذه الدراسة 20 بقرة حلوب من السلالة المحلية المختلطة تم تقسيمها إلى أربع مجموعات تتكون كل منها من خمس أبقار؛ (مجموعة 1): سليمة سريرياً وسلبية لاختبار كاليفورنيا (CMT) كمجموعة ضابطة سلبية، (مجموعة 2): مصابة بالتهاب الضرع وموجبة لـ CMT كمجموعة ضابطة موجبة، (مجموعة 3): مصابة بالتهاب الضرع وموجبة لـ CMT وأُعطيت جرعة علاجية من السيفكوينوم وحده، (مجموعة 4): مصابة بالتهاب الضرع وموجبة لـ CMT وعولجت بجرعات علاجية من السيفكوينوم والديفينهيدرامين. أظهرت النتائج عزل المكورات العنقودية الذهبية السالبة لإنزيم التجلط، والمكورات العنقودية الذهبية الموجبة لإنزيم التجلط، والعقديات أجالاكتيا، والإشريكية القولونية من الحالات المصابة سريرياً بالتهاب الضرع. في دراسة استنفاد المتبقيات، ظل السيفكوينوم قابلاً للكشف في حليب الأبقار المعالجة حتى اليوم العاشر من العلاج (0.006±0.002 ميكروجرام/مل)، رغم أنه كان أقل من الحد الأقصى المسموح به للمتبقيات (20 ميكروجرام/كجم). في الدراسة الديناميكية الدوائية، أدى العلاج بالسيفكوينوم-ديفينهيدرامين إلى انخفاض معنوي في الزيادات المرتبطة بالتهاب الضرع في تركيزات MDA و IgE, والهيستامين في المصل، مع الحفاظ على معايير وظائف الكبد الطبيعية، كما يتضح من عدم تغير نشاط AST و ALT في المصل. يمكن استنتاج أن العلاج بالسيفكوينوم-ديفينهيدرامين يُظهر فعالية علاجية متفوقة على العلاج الأحادي بالسيفكوينوم في مرض التهاب الضرع في الأبقار، كما يتضح في التعديل المحسن لعلامات الالتهاب وتحسن حالة مضادات الأكسدة.

الكلمات الدالة: التهاب الضرع، بقايا المضادات الحيوية، سيفكوينوم، ديفينهيدرامين.