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Pharmacokinetics and pharmacodynamics of intramammary cefquinome in lactating goats with and without experimentally induced *Staphylococcus aureus* mastitis

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Abstract

Values for pharmacokinetic variables are usually obtained in healthy animals, whereas drugs are frequently administered to diseased animals. This study investigated cefquinome pharmacokinetics in healthy goats and goats with experimentally induced mastitis. Five adult lactating goats received 75 mg of cefquinome intramammary infusion using a commercially available product into one udder half in healthy goats and goats with clinical mastitis that was induced by intracisternal infusion of 100 cfu of *Staphylococcus aureus* ATCC 29213 suspended in 5 ml of sterile culture broth. Cefquinome concentrations were determined in plasma and skimmed milk samples using high-performance liquid chromatography (HPLC). Pharmacodynamics was investigated using the California Mastitis Test and pH of milk. Experimentally induced mastitis significantly increased the California Mastitis Test score and pH, and decreased the maximal cefquinome concentration and shortened the half-life in milk when compared to healthy goats. In conclusion, mastitis facilitated the absorption of cefquinome from the mammary gland of lactating goats and induced marked changes in milk pH, emphasizing the importance of performing pharmacokinetic studies of antimicrobial agents in infected animals.

KEYWORDS
cefquinome, goats, intramammary, mastitis

1 | INTRODUCTION

Mastitis is a common and potentially serious health problem of lactating goats that can result in permanent damage to the udder. *Staphylococcus aureus* is the most common cause of clinical mastitis with gangrenous presentations and systemic signs of illness in small ruminants (Constable, Hinchcliff, Done, & Gruenberg, 2016; Ribeiro et al., 2007). The development of an effective treatment for goats with *S. aureus* mastitis would therefore be beneficial.

Cefquinome is a fourth-generation aminothiazolyl cephalosporin that was developed solely for veterinary use. The zwitterion molecular structure of cefquinome facilitates penetration of the outer plasma membrane of Gram-negative bacteria in a pH-dependent manner, and the introduction of a methoxyimino-aminothiazolyl moiety into the acyl side chain conferred resistance to inactivation by β-lactamases (Dołhań, Jelińska, & Bębenek, 2014). Cefquinome is considered a time-dependent antimicrobial agent with a low in vitro postantibiotic effect against *S. aureus* (Ahmad et al., 2015). Cefquinome has been approved for the treatment of acute mastitis, respiratory tract diseases, and foot rot in cattle, calf septicemia, respiratory diseases in pigs, and metritis–mastitis–agalactia syndrome in sows in Europe and elsewhere but not in the United States (CVMP,

2003; Gue´rin-Fauble´e, Carret, & Houffschmitt, 2003; Uney, Altan, & Elmas, 2011). Cefquinome has a wide spectrum of antimicrobial activity and is stable against chromosomally and plasmid-encoded beta-lactamase production (Limbert et al., 1991). To limit the selection of resistant bacteria, the European Medicines Agency has recommended that fourth-generation cephalosporins should be reserved for use where susceptibility testing indicates that alternatives are not available.

An intramammary (IMM) formulation of cefquinome sulfate (Cobactan LC) is commercially available in many countries and offers a convenient option for the treatment of clinical mastitis in lactating dairy cows. The IMM formulation attains and maintains an effective drug concentration at the site of infection in lactating dairy cows (Zonca et al., 2011); however, the IMM formulation of cefquinome sulfate has not been investigated as a potential treatment of *S. aureus* mastitis in goats. The labeled IMM cefquinome dose for lactating dairy cows is three infusions at 12-hr intervals but a labeled dose has not been established for lactating goats.

Values for pharmacokinetic parameters are usually obtained in healthy animals, whereas drugs are frequently administered to diseased animals. There is therefore an urgent need to determine whether dosage protocols need to be altered in sick animals. Accordingly, the objective of the study reported here was to identify the differences in the cefquinome concentration–time profile and pharmacokinetics in plasma and milk after IMM administration in goats with and without experimentally induced acute *S. aureus* mastitis. High-performance liquid chromatographic (HPLC) techniques are preferred for determination of antimicrobial withdrawal times for meat and milk after treatment (Thal et al., 2011).

2 | MATERIAL AND METHODS

2.1 | Drugs

Cefquinome IMM suspension (Cobactan LC®; INTERVET) was administered to animals, and cefquinome sulfate powder (Intervet Deutschland GmbH) was used as a standard for analytical purposes. Each IMM infusion tube contains 88.8 mg of cefquinome sulfate (equivalent to 75 mg cefquinome) in a total of 8 g of formulation. Cefquinome sulfate powder was dissolved in sterile 0.9% NaCl solution to produce a cefquinome sulfate concentration of 2.5% for stock solution for preparation of a standard curve.

2.2 | Animals

Five adult clinically healthy lactating Zaraibi (Egyptian Nubian) goats in early lactation to mid-lactation, weighing 20–22 kg and aged from 30 to 36 months, were obtained from local farms. Goats were housed together at Cairo University in one large indoor stall and fed Berseem clover (*Trifolium alexandrinum*, also known as Egyptian clover) and concentrates with free access to food and water. Goats were milked by hand once a day at approximately 9 a.m. Daily milk production ranged from approximately 1.0 to 1.5 L/goat. The bacteriologic status of the mammary glands was determined by milk cultures using National Mastitis Council (1999) guidelines on two consecutive days after arrival. Glands used in the study showed no growth on both cultures.

2.3 | Experimental design

The protocol was approved by Institutional Animal Care and Use Committee of Cairo University (IACUC). On the day of drug administration, the left and right jugular furrows were shaved, cleaned, and sterilized with tincture of iodine (2.5%). The five mastitis free goats received a single IMM infusion. Clinical mastitis was induced 2 weeks later in the same goats by infusing 100 cfu of *S. aureus* ATCC 29213 suspended in 5 ml of sterile culture broth into single teat (study half) immediately following the morning milking (Erskine, Eberhart, & Scholz, 1990). The rectal temperature was obtained each morning using a digital thermometer (Kruuse Digi-Vet SC 12 digital thermometer). The somatic cell count in the glandular secretion was monitored once daily using the California Mastitis Test (CMT; Schalm & Noorlander, 1957) before milking. The severity of clinical mastitis was graded as described elsewhere (Morin, Shanks, & McCoy, 1998), with 0 = normal, 1 = abnormal milk, 2 = abnormal milk and gland, and 3 = abnormal milk, gland, and systemic signs of illness.

Milk samples were collected for bacteriologic culture before initiating cefquinome treatment when an increase in CMT score and clinical signs of mastitis were evident, such as presence of flakes, clots, or blood in the secretion (score = 1), heat, swelling, redness, and pain in the mammary gland (score = 2). The teat and teat orifice were thoroughly cleaned and disinfected with the cleaning towel provided, the cap of an IMM infusion tube containing 75 mg of cefquinome was removed, and the infusion tube cannula partially inserted into the teat canal. Partial insertion was used because it caused less disruption of the keratin lining of the streak canal which represents the udder's first line of defense to infection (Shearer & Harris, 2003). The contents of one IMM infusion tube (8 g of formulation containing 75 mg of cefquinome) were then gently infused into the mastitic udder half followed by dispersing of antibiotic via gentle massage of the teat and udder.

Milk samples were obtained before and at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 hr (5 days), as well as 168 (7 days) postcefquinome administration. At each time, the first two streams of milk from the udder half were discarded and approximately 6 ml of secretion was then removed from the infused udder half. The total volume removed at each sampling time was <10 ml, which represented <2% of the estimated milk volume in the udder half; the sample was therefore thought to reflect the cefquinome concentration in gland cistern milk (Stockler, Morin, Lantz, & Constable, 2009).

Milk samples were collected into clean sterile tubes in duplicate. One aliquot was centrifuged at 3,000 *g* for 15 min; the fat-free liquid was then aspirated and stored a −70◦C until analyzed within 3 months for determination of cefquinome concentration. The fat-free portion of the milk aliquot was collected for analysis because it was believed to more closely resemble the concentrations within the gland as a whole

than does the milk fat layer due to cefquinome hydrophilic properties (Behbood, Mrestani, Warrass, & Neubert, 2017).

Blood samples (5 ml) were collected from the right jugular vein of each goat immediately before medication and at intervals of 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 hr (5 days) after drug administration. Blood samples were collected into 6-ml clean sterile centrifuge tubes containing heparin, centrifuged at 3,000 *g* for 15 min, and 2 ml of plasma harvested and stored at −70°C until analyzed within 3 months for cefquinome concentration.

2.4 | Analytical procedure

2.4.1 | High-performance liquid chromatography

Cefquinome was assayed at room temperature using minor modification of previously described HPLC methodology (McCormmick, Echols, & Rosano, 1984; Yu et al., 2008) as described elsewhere (El Badawy, Amer, Kamel, Eldeib, & Constable, 2015). Separation was done using symmetry c18 Column 4.6 × 250 mm flow-T90161E 19 (Fortis Technologies Ltd). A 5 cm guard column packed with CO:PELL ODS was placed between the injector and the analytical column. The mobile phase consisted of 130 ml of acetonitrile with 28 ml glacial acetic acid diluted to final volume of 1,000 ml with ultrapure analytical grade type 1 water for HPLC that was produced using a water purification system (Millipore). The flow rate was 1.5 ml/min and variable wavelength UV absorbance detector was set at 254 nm.

The LOQ of the used HPLC assay was 0.017 μg/ml and 0.018 μg/ml in skimmed milk and plasma, respectively, while LOD 0.006 μg/ml was in both skimmed milk and plasma (El Badawy et al., 2015). The coefficient of variation (CV) at low (0.7 μg/ml), moderate (6.25 μg/ml), and high (25 μg/ml) cefquinome concentrations was calculated by spiking plasma and skimmed milk samples from healthy and mastitic goats using HPLC as described (El Badawy et al., 2015).

2.5 | Assessment of severity of clinical mastitis

The severity of clinical mastitis was evaluated using the scoring system, the California Mastitis Test, and by determining pH in the glandular secretion. Somatic cell count was categorized using the California Mastitis Test (CMT) as adapted for use in lactating goats ((Escobar, 1995); Table 1). Somatic cell counts in excess of 1,500,000/ml indicate IMM infection in goat milk (Haenlein, 2002). Glandular secretion pH was measured at room temperature (25°C) using a PICCOLO plus pH meter (HANNA instruments) that was calibrated using pH 4.0 and pH 7.0 buffers.

2.6 | Pharmacokinetic analysis

A noncompartmental approach was applied to determine the PK parameters separately in each goat (with or without mastitis) using a software program (WinNonLin, Pharsight). The area under the curve (AUC) and the area under the first moment curve (AUMC) were calculated for healthy and mastitic goats from the cefquinome concentration–time relationship using the trapezoidal method, with the area to the last time point.

The extent of cefquinome absorption after IMM infusion was calculated by comparing the area under the plasma concentration curve

TABLE 1 California Mastitis Test results, rectal temperature, and mastitis grade (0 to 3) of lactating goats (n = 5) before and after experimental induction of *Staphylococcus aureus* mastitis following IMM infusion of cefquinome (75 mg) into single study udder half

	CMT					
Time (hr)	CMT score	Description	Interpretation, cells/ml	Rectal temperature (°C)	Mastitis grade	Milk pH
Before infection	Trace	Slight slime disappear with swirling	150,000–500,000	38.5 ± 0.2	0	6.32 ± 0.41
After infection	3+	Gel develop convex surface and adhere to the bottom of the cup	Over 5,000,000	40.0 ± 0.2**	3	7.20 ± 0.20
After IMM infusion 1	3+			ND	3	7.18 ± 0.29
2, 4	3+			ND	3	6.96 ± 0.18
6, 8	2+	Immediate gel formation moves as a mass with swirling	800,000–5,000,000	ND	3	6.94 ± 0.16
12	2+			ND	3	6.92 ± 0.19
24	2+			39.1 ± 0.1**	2	6.32 ± 0.07
48	2+			38.8 ± 0.1*	2	6.60 ± 0.11
72	1+	Distinct slime but without gel formation	400,000–1,500,000	38.4 ± 0.2	1	6.54 ± 0.16
96	1+			38.4 ± 0.1	1	6.52 ± 0.27
120	1+			38.4 ± 0.1	1	6.36 ± 0.10

Note: Rectal temperature and milk pH are expressed as mean ± *SD*.
Abbreviation: ND, not detected.
*$p < 0.05$,
**$p < 0.005$ compared to healthy goats.

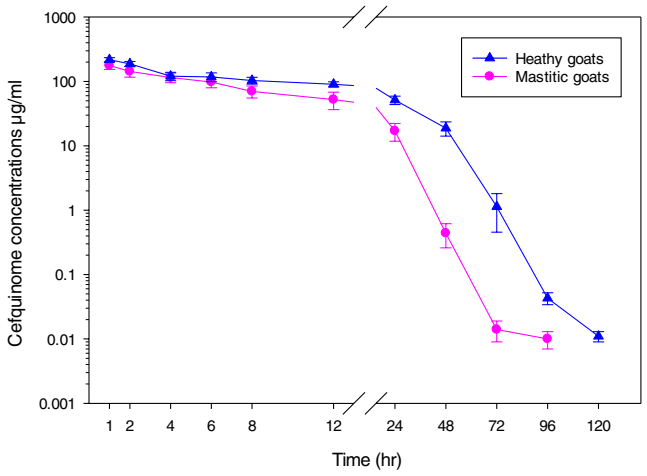

FIGURE 1 Semilogarithmic graph of the cefquinome–time relationship in skimmed milk after a single intramammary infusion of cefquinome (75 mg) in healthy and mastitic lactating goats. Cefquinome concentration was measured using HPLC (mean ± *SD*, *n* = 5) [Colour figure can be viewed at wileyonlinelibrary.com]

to that obtained when the same dose was given intravenously to the same goats using a standard equation (Toutain & Bousquet-Melou, 2004) as $F = AUC_{IMM} \times 100/AUC_{IV}$ (%). Values of AUC_{IV} following IV administration at the same dose were obtained in our previous study (El Badawy et al., 2015). Mean residence time (MRT) was calculated from the ratio of AUMC to AUC. Mean absorption time (MAT), mean transit time (MTT), absorption rate constant (k_{ab}), absorption half-life ($T_{½ab}$), and the elimination rate constant (K_{el}) were calculated using the following standard equations: $MAT = MRT_{IMM} - MRT_{IV}$ (hr), MTT = MRT + MAT (hr); $k_{ab} = 1/MAT$ (1/hr), $T_{½ab} = 0.693 \times MAT$ (hr).

The apparent plasma half-life ($T_{1/2}$) was calculated as $T_{1/2} = 0.693 \times MRT$. The elimination rate constant k_{el} was calculated as k_{el}, 1/MRT. The time to reach peak concentration (t_{max}) following IMM administration of cefquinome was calculated using the following equation (Baggot, Powers, Powers, Kowalski, & Kerr, 1978): $t_{max} = 2.303 \times \log_e(k_{ab}/k_{el})/(k_{ab} - k_{el})$ (hr), where $\log_e$ is the natural logarithm.

The time that the cefquinome concentration remained greater than the maximum residue level (MRL) for the European Union (t > MRL = 0.020 µg/ml; Nouws et al., 1999) was determined from the cefquinome concentration–time curves in skimmed milk and plasma following IMM administration. The mean time to MRL is not the regulatory method for determining withdrawal times, so these data should not be used to validate withdrawal time estimates.

2.7 | Statistical analysis

Linear regression was applied to standard curve data using a software program (Excel, Analysis ToolPak add-in, Microsoft Office 14, Microsoft Corp). Data were expressed as mean ± *SD*, and $p < 0.05$ was considered significant. Repeated-measures ANOVA was used to determine the main effects of infection status (healthy or mastitic), time, and the interaction between infection status and time, using mixed models procedure and an autoregressive(1) covariance matrix (SAS 9.3, SAS Inc). The mixed linear model procedure (PROC MIXED), restricted maximum-likelihood (REML) estimation method, and a compound symmetry covariance matrix were used for this analysis.

3 | RESULTS

3.1 | Induction of clinical mastitis

All goats became pyrexic by 24 hr after infusion of *S. aureus* into the udder half, at which time clinical mastitis was present, characterized by an abnormal secretion with visible clots and an inflamed udder half as indicated by swelling, increased local temperature and pain on palpation (clinical score = 3). The secretion of infused udders at 24 hr after infusion of *S. aureus* had a 3+ score for CMT and a marked increase in milk pH.

3.2 | Pharmacodynamics of cefquinome post-IMM infusion

Milk pH increased markedly after induction of infection then started to decrease at 24 hr post-IMM infusion of cefquinome to reach 6.4 at 120 hr post-IMM infusion (Table 1). Mean milk pH in healthy goats was 6.51 at 1 hr postinfusion and decreased slightly over time postinfusion reaching 6.34 at 120 hr. Mean secretion pH increased to 7.20 after induction of *S. aureus* infection. Following cefquinome infusion, the mean secretion pH value decreased gradually until reaching 6.36 at 120 hr, at which time there were no significant differences between healthy and mastitic goats. Changes in somatic cell count following IMM infusion of cefquinome in mastitic goats are shown in Table 1.

3.3 | Pharmacokinetics of cefquinome following intramammary infusion

Mean skimmed milk and plasma cefquinome concentration–time relationships determined by HPLC for healthy goats and goats with experimentally induced mastitis following a single cefquinome IMM infusion of 75 mg into study half (Cobactan LC®) are presented in Figures 1 and 2. The pharmacokinetic variables that describe the disposition of cefquinome in skimmed milk and plasma following a single cefquinome IMM infusion of 75 mg into an udder half are presented in Table 2. Cefquinome was detected in milk until 120 and 96 hr after IMM infusion in healthy animals and infected animals, respectively, with significantly lower concentrations (0.010 ± 0.002 µg/ml) than the corresponding concentrations at 96 hr for healthy goats (0.043 ± 0.009 µg/ml).

Following single IMM infusion, cefquinome was detected in plasma at 4 hr and 1 hr postinfusion in healthy goats and goats after experimental induction of *S. aureus* mastitis, respectively.

The T > MRL in skimmed milk was 110.0 ± 3.3 hr in healthy goats, while the T > MRL in mastitic goats was shorter 68.4 ± 5.2 hr.

Assuming the 24-hr interdose interval, the %T > MIC in skimmed milk following a single IMM infusion was 330 ± 34% in healthy goats and 249 ± 4% in mastitic goats, respectively.

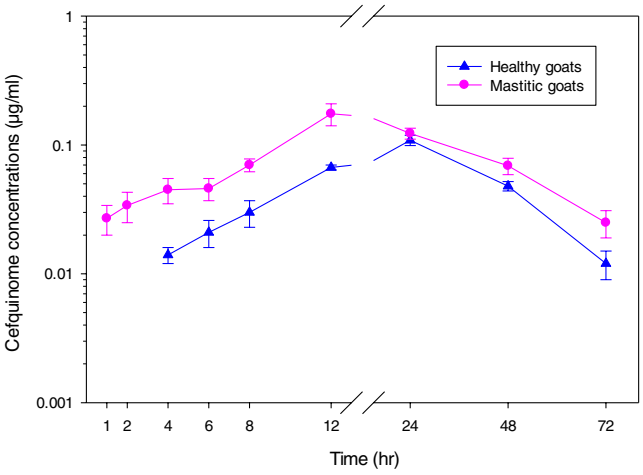

FIGURE 2 Semilogarithmic graph of the cefquinome–time relationship in plasma after single intramammary infusion of cefquinome (75 mg) in healthy and mastitic lactating goats. Cefquinome concentration was measured using HPLC (mean ± *SD*, *n* = 5) [Colour figure can be viewed at wileyonlinelibrary.com]

Cefquinome concentration in plasma never exceeded the MIC in healthy nor mastitic goats following IMM infusion. The T > MRL in plasma of healthy goats was 76.0 ± 4.9 hr, while in mastitic goats, a shorter T > MRL of 64.8 ± 2.5 hr was observed. There was a marked difference in the values for cefquinome pharmacokinetic variables in skimmed milk and plasma between healthy and mastitic goats (Table 2).

4 | DISCUSSION

Pharmacokinetic studies are usually carried out in healthy animals whereas antimicrobial drugs are administered to diseased animals, where drug behavior can be modified by various factors (Mestorino & Errecalde, 2012). The results of the study reported here clearly demonstrate important differences in the pharmacokinetic values in skimmed milk and plasma between healthy and mastitic goats that associated with changes in residue risk. When taken into consideration with the results of other studies (El Badawy et al., 2015; Cagnardi et al., 2014, 2010; Gehring & Smith, 2006; Goutalier, Combeau, Quillon, & Goby, 2012; Owens, Xiang, Ray, & Nickerson, 1990; Zonca et al., 2011), serious concerns must be raised about the clinical value of pharmacokinetic values such as C_{max}, AUC, and K_{el} determined in healthy animals for drugs that are intended to be

TABLE 2 Cefquinome pharmacokinetic parameters in skimmed milk and plasma after a single intramammary infusion of cefquinome (75 mg per udder half) in lactating goats before and after induction of *Staphylococcus aureus* mastitis

	Skimmed milk		Plasma	
Parameters	**Healthy goats**	**Infected goats**	**Healthy goats**	**Infected goats**
C_0 (µg/ml)	252.5 ± 17.1	220.6 ± 21.2*	0.014 ± 0.002	0.027 ± 0.005**
Actual C_{max} (µg/ml)	217.8 ± 16.3	177.4 ± 19.1*	0.109 ± 0.010	0.175 ± 0.024**
Actual T_{max} (hr)	1 ± 0	1 ± 0	24 ± 0	12 ± 0
Calculated T_{max} (hr)			28.5 ± 0.3	27.4 ± 0.7*
K_{ab} (/hr)			0.037 ± 0.001	0.039 ± 0.001
$T_{½ab}$ (hr)			18.6 ± 0.2	18.0 ± 0.5
T_{last} (hr)	120 ± 0	96 ± 0		
$T_{1/2}$ (hr)	11.8 ± 0.4	6.4 ± 0.1*	18.6 ± 0.2	26.6 ± 3.0*
k_{el} (/hr)	0.059 ± 0.002	0.109 ± 0.002**	0.029 ± 0.001	0.026 ± 0.003*
AUC_{last} (µg ml hr^{-1})	3,544 ± 219	1,876 ± 207**	4.3 ± 0.3	6.9 ± 0.4**
$AUMC_{last}$ (µg ml hr^{-2})	60,351 ± 3,288†	17,320 ± 2,164**	147 ± 12	267 ± 41**
MRT_{last} (hr)	17.0 ± 0.6	9.2 ± 0.16**	34.5 ± 1.5	38.3 ± 4.4
MAT (hr)			26.8 ± 0.3	26.0 ± 0.7
MTT (hr)			61.3 ± 1.8	64.4 ± 5.0
F (%)			9.6 ± 0.6	34.4 ± 3.8**

Note: Cefquinome concentration was assessed using high-performance liquid chromatography (mean ± *SD*, *n* = 5).
Abbreviations: AUC, area under the plasma concentration–time curve from time 0 to last time point; AUMC, area under the moment curve from time 0 to last time point; C_0, plasma concentration at time 0; C_{max}, maximal plasma concentration; F, bioavailability; k_{ab}, rate constant for absorption; k_{el}, rate constant for elimination from the central compartment; MAT, mean absorption time; MRT, mean residence time from time 0 to last time point; MTT, mean transient time; $T_{1/2}$, the apparent half-life (for i.mm. route); $t_{1/2ab}$, apparent absorption half-life; Tlast, time of last measurement; T_{max}, time of occurrence of C_{max}.
*$p < 0.05$,
**$p < 0.005$ compared to healthy goats for the same assay.

administered to diseased animals. This highlights the need for performing pharmacokinetic studies in diseased animals.

Lower concentrations of cefquinome were observed in skimmed milk of mastitic than healthy goats following IMM administration of 75 mg into one udder half. Our finding was consistent with previous reports for milk cefquinome concentrations of 138 ± 48 and 172 ± 41 μg/ml in mastitic and healthy cows, respectively (Zonca et al., 2011), milk cefoperazone concentrations of 310 ± 181 and 446 ± 287 μg/ml in mastitic and healthy cows, respectively (Cagnardi et al., 2010), and milk cephapirin concentrations of 235 ± 141 and 1,335 ± 1,323 μg/ml in mastitic and healthy cows, respectively (Cagnardi et al., 2014). We attribute part of the consistently lower concentrations of cefquinome found in milk after IMM treatment to breakdown in the blood–milk barrier.

Systemic cefquinome absorption was negligible in healthy and mastitic animals although it was higher in mastitic than in healthy goats. The increased absorption is most likely due to damage of epithelial cell junctions caused by inflammation (Cagnardi et al., 2010; Zonca et al., 2011). Cefquinome was detected in milk until 96 hr in mastitic goats and 120 hr in healthy animals. A similar pattern of shorter T_{last} in mastitic than healthy animals was reported for cefquinome in cows (Zonca et al., 2011) and cephapirin in cows (Cagnardi et al., 2014).

A noncompartmental analysis was applied to the concentration–time data for each goat in skimmed milk and plasma following IMM infusion an IV bolus model as previously reported (Stockler et al., 2009) and extravascular model, respectively. The noncompartmental analysis was used for the concentration–time data following IMM infusion by other investigators (Cagnardi et al., 2014; Zonca et al., 2011). Based on concentrations over time, the pharmacokinetics of cefquinome in milk from healthy goats was characterized by significantly greater area under concentration–time curve AUC and AUMC than in infected animals. A similar result was reported for cefquinome in cow's milk (Zonca et al., 2011), cephapirin in cow's milk (Cagnardi et al., 2014), and cefoperazone in cow's milk (Cagnardi et al., 2010). The variable degree of variation in the reduction of AUC as a result of infection is probably related to the severity of infection and consequently the disruption in mammary epithelial lining, thus confirming the uneven distribution of drugs in infected goats, as observed in previous studies (Cagnardi et al., 2010; Goutalier et al., 2012; Zonca et al., 2011).

The mean half-life ($T_{1/2}$) in skimmed milk was shorter in mastitic goats than in healthy goats. In contrast, reduction of half-lives of cefquinome, cephapirin, and cefoperazone between mastitic and healthy animals was smaller in goats than in cows (Cagnardi et al., 2014; Li et al., 2008; Zonca et al., 2011). The difference in results was most likely due to the higher volume of milk production in cows compared to goats that favors dilution of the antimicrobial and therefore a smaller flux across the milk blood barrier. The effect of milk volume on pharmacokinetics in milk has been documented (Rule, Cordiviola, Vita, & Lacchini, 2004; Whittem, 1999; Ziv, 1980), and a shorter half-life for cefquinome has been observed in milk from mastitic quarters in cows 4.2 hr (Zonca et al., 2011), cephapirin 4.8 hr (Cagnardi et al., 2014), and cefoperazone 4.3 hr (Cagnardi et al., 2010). The shorter half-life in mastitic quarters is more likely due to increased systemic absorption because of disruption to epithelial cell junctions (Cagnardi et al., 2010; Elmas, Yazar, Uney, & Er Karabacak, 2006) rather than increased rate of renal excretion (CVMP, 1995).

The MIC of cefquinome against *Staphylococcus aureus* ATCC 29213 is 0.25 μg/ml (Wang, Shan, Ding, Liang, & Zeng, 2014) and ranges from 0.06 to 0.39 μg/ml against *Escherichia coli*, *Pasteurella multocida*, *and Streptococcus agalactia* (Chin, Gu, Fang, & Neu, 1992; Dumka, Dinakaran, Ranjan, & Rampal, 2013; Murphy, Erwin, & Jones, 1994; Orden, Ruiz, Garcia, Cid, & Fuente, 1999; Sheldon, Bushnell, Montgomery, & Rycroft, 2004; Thomas, Thomas, & Wilhelm, 2006). After single cefquinome IMM infusion (75 mg/udder half), mean values of T > MIC in skimmed milk were shorter in mastitic goats compared with healthy goats—57 and 81 hr, respectively. A similar result was recorded for cefquinome in mastitic cows compared with healthy cows—42 and 54 hr, respectively (Zonca et al., 2011), and for cephapirin in mastitic cows compared with healthy cows—35 and 38 hr, respectively (Cagnardi et al., 2014). In contrast, T > MIC of cefoperazone was longer in mastitic than healthy cows, at 65 and 58 hr, respectively (Cagnardi et al., 2010). The T > MIC may be helpful to compare the data but the concentration above the MIC did not confirm clinical efficacy.

Chemical modifications of the basic cephalosporin structure provide cefquinome zwitterionic property that facilitates rapid penetration across biological membranes in addition to C3 bicyclic pyridinium structure that accounts for its resistance to β-lactamase.

Cefquinome may therefore find more future use as it has excellent in vitro activity and favorable pharmacokinetic properties compared to other cephalosporin antibiotics (Al-Taher, 2010; Chin et al., 1992; Wang et al., 2014). However, in vitro activity is not necessarily predictive of clinical or bacteriologic cure rate of mastitis with IMM cephapirin administration (Constable & Morin, 2002).

The health of udder halves of goats before induction of infection was confirmed by the presence of a CMT score of zero using test interpretations for goat milk (Haenlein, 2002). A marked increase in somatic cell count (SCC) was present based on a 3+ CMT score at 24 hr post-*S. aureus* infusion, equivalent to a SCC > 5,000,000 cells/ml (Haenlein, 2002; Stuhr & Aulrich, 2010). The increased CMT score primarily reflects the migration of neutrophils from blood into the glandular secretion in response to infection (Zhao & Lacasse, 2008). Following IMM infusion, the SCC decreased gradually to reach 400,000 to 1,500,000 cells/ml (+1 score) at 72 hr post-IMM of cefquinome with no further reduction afterward, using the interpretation for goats (Haenlein, 2002), although SCC at 72 to 104 hr was still higher than before induction of infection (150,000–500,000 cell/ml), suggesting partial resolution of inflammation and phagocytosis of dead cells (Viguier, Arora, Gilmartin, Welbeck, & O'Kennedy, 2009).

The mean milk pH in healthy goats was 6.3, consistent with pH values of 6.5 ± 0.1 reported elsewhere for milk from healthy goats (Morgan et al., 2003). Mean milk pH values increased to 7.2 after

experimental induction of *S. aureus* mastitis; the increase in milk pH has been attributed to an increase in the difference between the concentration of the main strong cation in milk (sodium) and the concentration of the main strong anions in milk (chloride and casein), thereby increasing the milk strong ion difference and pH (Kandeel, Megahed, Ebied, & Constable, 2019). An increased milk pH should cause more drug ionization and thus decreased diffusion across the blood–milk barrier, which was the opposite what we observed. Disruption of the blood–milk barrier is therefore the main factor favoring systemic absorption in mastitis (Cagnardi et al., 2010).

It is important to note that because we did not have an untreated control group in the study reported here we cannot claim that IMM cefquinome was an efficacious treatment for experimentally induced *S. aureus* mastitis in goats. Efficacy is supported by the results of studies in dairy cows, demonstrating that IMM cefquinome is efficacious when infused in cows with coliform mastitis (Shpigel et al., 1997), *Streptococcus uberis* mastitis (Milne et al., 2005), caused by coagulase-negative staphylococci, environmental streptococci, and coliforms (Kasravi et al., 2011), *S. aureus* mastitis (Swinkels, Cox, Schukken, & Lam, 2013), and a number of mastitis pathogens (Bradley & Green, 2009).

5 | CONCLUSIONS

The single intramammary administration of cefquinome at the dose of 75 mg/udder half in lactating goats resulted in higher drug concentrations in milk of healthy goats than in milk from goats with experimentally induced mastitis. According to our results, the IMM infusion of 37.5 mg cefquinome/udder half twice at a 48-hr interval is suggested to be efficacious against *S. aureus* ATCC 29213 mastitis, but clinical efficacy studies are required to confirm this supposition.

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CONFLICT OF INTEREST

None of the authors of this paper has a financial or personal relationship that could inappropriately influence or bias the content of the manuscript.

AUTHOR CONTRIBUTIONS

Shymaa El Badawy contributed to study design, execution, data analysis, and interpretation, in addition to manuscript preparation. Aziza Amer and Peter Constable contributed to the study design, data analysis, and gave final approval of the manuscript. Gehan Kamel and Kamal Eldeib contributed to technical assistance.

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