



Assessment of ceftiofur residues in cow milk using commercial screening test kits

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ABSTRACT

Ceftiofur, a third-generation cephalosporin, is one of the most used antibiotics in dairy industry. Intramuscular injection of 1 mg/kgBW ceftiofur hydrochloride (HCl) generally results in 0 hour withdrawal time for the milk in dairy cows. Nevertheless, farmers and dairy processors occasionally complain about ceftiofur-based products in case of positive result to a commercial rapid screening test for the presence of violative residues of antimicrobials (inhibitors) in the bulk milk tank. Six lactating cows were injected with a 50 mg/ml ceftiofur HCl-based product at the dosage regimen of 1 mg/kg, intramuscularly, once a day, for five consecutive days, as per label. Milk samples were then collected just before the very last injection (T_0) and then at 12, 24, 36, 48, 60, 72, 84 and 96 hours after the last injection. Individual milk samples were tested using three commercial screening test kits for inhibitor residues: DelvotestSP NT, SNAP Beta-Lactam ST Plus and ROSA MRL Beta-Lactam Test. Since bulk tank is screened in real operating conditions, samples were also diluted to 1:4, 1:10 and tested again. For the Delvotest SP NT, which lowest detected concentration is close the MRL of the ceftiofur (100 µg/kg), all results were negative. For the ROSA MRL Beta-Lactam Test and the SNAP Beta-Lactam ST Plus, several samples yielded positive and doubtful results at T_0 and T_{12} . However, after dilution to 1:10, all results were negative. Consequently, when used as officially instructed, the tested 50 mg/ml ceftiofur HCl-based injectable veterinary products are safe, and milk should be free of violative residues of ceftiofur. With consideration to the low specificity and the low positive predictive value of commercial screening tests, positive reactions of the bulk milk should be interpreted as false positive or another risky usage of β -lactam-based medicines in the farm must be investigated.

INTRODUCTION

The European Union (EU) requires by law that foodstuff such as meat, milk or eggs must not contain residue levels of veterinary medicines that might represent a hazard to the consumers' health. Before a veterinary medicine intended for food-producing animals is authorised in the EU, the Committee for Veterinary Medicinal Products (CVMP) evaluates the safety of its pharmacologically active substances and their residues and defines the maximum residue levels (MRLs) for the

different foodstuff (Directive 2001/82/EC). Such regulatory provisions exist in other regions worldwide.

Ceftiofur is a third-generation cephalosporin considered critically important antimicrobial according to the WHO.¹ It is one of the most used antibiotics in dairy cows around the world. By the parenteral route, ceftiofur is used as per label for the treatment of bacterial pneumonia in cattle, interdigital phlegmon, acute puerperal metritis^{2,3} and off-label for mastitis³ and some anecdotal conditions such as ischaemic teat necrosis.⁴ Intramammary medicines have also been developed for the treatment of clinical mastitis. Minimum inhibitory concentrations 90% (MIC_{90}) are deemed to be low or very low for most of the bacteria involved in the above conditions.⁵ In dairy cow, recommended dosages for the parenteral route (subcutaneously and intramuscularly) for ceftiofur sodium or hydrochloride range from 1 mg/kg to 2.2 mg/kg once a day.

After injection, ceftiofur is rapidly metabolised in desfuuroylceftiofur (DFC). Thanks to its pharmacokinetic (PK) features, ceftiofur is mainly excreted in urine and faeces. Only 0.15% of ceftiofur and DFC is excreted into the milk.⁶ Moreover, in healthy adult cows, ceftiofur metabolites are mostly (>90%) serum protein bound.⁷ This increases the excretion half-life ($T_{1/2}$) compared with most other cephalosporins and decreases the rate of excretion in milk. From a toxicological point of view, the non-observed effect level is 30 mg/kg in rats and dogs. With consideration to the Minimum Inhibitory Concentration 50% (MIC_{50}) of a selection of the most sensible bacterial species that populates the intestine of man, the acceptable daily intake (ADI) of ceftiofur has been set at 20 µg/kg/day, that is, 1200 µg/day for a 60 kg person. PK and toxicological features were taken into account by the responsible authorities to set the MRL to 100 µg/kg (100 ppb) for the milk of all agriculture mammals. For therapeutic dosages lower than 2.2 mg/kgBW in cattle,



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the residual concentration of DFC residues in cow milk is below the MRL. The recommended withdrawal period for lactating animals is nil; the milk of the treated animal may be sold for human consumption, provided that the milk composition also meets all the other quality criteria.

The determination of MRL and withdrawal period are largely misunderstood concepts. Zero-day milk withdrawal period is not clearly understood in the field, and it is often confused with a guarantee of absence of antibiotic residues or inhibitors. In a study on milk withheld for sale because of antibiotic treatment or high somatic cell count in New York upstate, ceftiofur was the most frequently detected β -lactam, present in 39.2% of the waste milk samples (mean \pm SE=151 \pm 42 μ g/l>MRL).⁸ Nowadays, in the progressive dairy industry, milk is routinely tested with rapid screening tests against microbial inhibitory substances before processing, in order to detect excessive residue levels of inhibitory substances. Positive results and related financial penalties result in complaints from the dairymen who used only ceftiofur-based medicines (regardless the conditions of use).

Additionally, for the test product of this study, it was substantiated that the ketoprofen positively influence the bioavailability of the ceftiofur.⁹ Compared with a similar commercial product free of ketoprofen, serum concentration of ceftiofur tended to be higher during the first 12 hours after injection, and the area under the curve between 0 hour and 24 hours after injection (AUC_{0-24h}) was 12.8% higher (72.2 \pm 11.9 v 63.9 \pm 17.1 μ g/hour/ml). Although mandatory safety studies evidenced the absence of residues beyond the 100 μ g/kg limit, we wondered whether the combination of the two actives could occasionally influence the reactions of rapid screening tests.

The objective of this study was to assess the risk of positive reaction to three widely used rapid screening test for inhibitors in milk from cows treated with a new ceftiofur/ketoprofen-based medicine at the recommended dosage.

MATERIAL AND METHODS

Animals

Six lactating Holstein cows were purchased from a commercial dairy operation in the province of Ferrara (Italy) in June 2018. Animals were selected at convenience, provided that they were correctly identified, healthy, free of clinical mastitis, without recent history of antibiotic treatment, if any, and with individual composite somatic cell count lower than 300 000 cells/ml at the last test day. Three of them were at an early stage of lactation and yielded more than 25 l milk per day (high yielding animals); the remaining three were at a late stage of lactation and yielded less than 25l/day (low yielding), in compliance with the *Note for guidance for the determination of withdrawal periods for milk* of the European Agency for the Evaluation of Medicinal Products (EMA/CVMP/473/98-FINAL).

Animals were subjected to veterinary clinical examination at the time of the enrolment, before the treatment

starts and during the animal phase of the study, whenever required. Particular attention was paid to the neck side areas (injection sites) and mastitis. Animals were housed for seven days before treatment in a barn with free access to water and feed. They were fed ad libitum on a ration that nominally contains no antibiotics, growth promoters or other non-nutritional additives and that met the needs of the animals. Water and feed consumption was not recorded. All cows were milked out twice a day.

Treatment

The test product was an injectable suspension of ceftiofur hydrochloride (ceftiofur HCl) (50mg/ml) and ketoprofen (150mg/ml) (Curacef Duo, Virbac, Mexico). All six animals were dosed according to their live weight (705, 670, 605, 560, 512 and 523kg) at the dosage regimen of 1 ml/50kg (14.5, 13.5, 12.5, 11.5, 10.5 and 10.5 ml, respectively), intramuscularly, once a day, for five consecutive days, as per label. This dosage corresponds to a dose of 1 mg/kgBW and 3 mg/kgBW of ceftiofur and ketoprofen, respectively.

Sampling regimen

Milk samples were collected from the cows within 2 hours before the first injection ($T_{baseline}$) in order to check whether milk of the experimental animals was free of inhibitory residues. Samples were then collected before the very last injection (T_0) of the test product, and then at 12, 24, 36, 48, 60, 72, 84 and 96 hours ($T_h \pm 30$ min) after the last injection. Samples were identified and frozen for further analysis.

Screening test kits

To assess the presence of inhibitor residues in the milk, all milk samples were tested using three commercial screening test kits, Delvotest SP NT (Delvotest) in ampoule format (DSM Nutritional Products, Fidenza, Italy), SNAP Beta-Lactam ST Plus (SNAP test, Idexx Laboratories Italia Srl, Milano, Italy), and ROSA MRL Beta-Lactam Test (ROSA test, Charm Sciences Inc, Lawrence, Massachusetts, USA).

Delvotest is designed to test every kind of milk for the presence of antibacterial substances, such as antibiotics. The test is made of an agar gel containing bacterial spores of *Bacillus stearothermophilus* var. *calidolactis*, and a colour indicator. The test takes 3 hours. Delvotest SP NT is granted a *Performance Tested Method*SM certification.¹⁰ Delvotest is recommended for tank milk testing, before delivery, and testing of individual milk whenever an antibiotic treatment has been applied and can extend the withholding period. The use of several treatments at the same time or a too short dry period are examples of occasions where testing is advised.

SNAP test is an enzyme-linked, receptor-binding assay in which β -lactams are captured by a binding protein on a solid support adsorbent matrix housed in a moulded plastic unit. The assay procedure includes three simple steps with a total assay time of less than 10 min for a sample.

ROSA test (for Rapid One Step Assay) is an immunoreceptor assay using a lateral flow technology that detects three primary β -lactam drugs in milk at or below EU MRL. The total testing time is 3 min.

All tests were used in accordance to manufacturer's instructions in conditions similar to those observed in progressive dairy farms or in dairy plants. Doubtful results, if any, were tested again for confirmation.

The dilution of the samples that yielded a positive result (or doubtful result confirmed positive) was performed to mimic the common condition observed in dairy farms where the dilution of individual animal milk occurs when adding it into the herd bulk milk. Milk was firstly diluted to 1:4 to mitigate the influence of non-antibiotic inhibitory substances. In case of positive results, samples were then diluted to 1:10 in order to get closer to the real conditions in dairy operations (≥ 10 lactating dairy cows).

Cohen's κ coefficient was calculated for every pair of tests used in order to evaluate agreement between tests. Only the first two days after the treatment were considered at higher risk of positive samples, so the agreement between tests was determined for the first four milking. Level of agreement was then interpreted using the Landis-Koch scale.¹¹

RESULTS

Screening tests

Results of screening tests are presented in tables 1–3; results are presented as negative (–, no reaction), positive (+, positive reaction) or doubtful (d, no decision). Confirmation results are presented in brackets. Only results for the first 24 hours after the last injection are presented because all results beyond 24 hours were negative.

For the Delvotest, all results were negative, after nullification of a doubtful reaction (table 1). Therefore, concentration of ceftiofur and its metabolites was very likely to be less than 100 $\mu\text{g}/\text{kg}$ during the treatment course (all T_0 results are negative) and during the withholding time consequently.

For the ROSA test (table 2), several samples yielded positive and doubtful confirmed positive results at T_0 (last injection), T_{12} (first milking after the last injection) and up to T_{24} . For the SNAP test (table 3), several positive results were reported on T_0 and T_{12} . All samples were found negative at all other control points.

Screening tests after dilution

All samples that tested positive were first diluted with three parts (v/v) of commercial milk, presumably free of ceftiofur residues. All results were negative but one from cow #5. This sample tested d for the ROSA test and + for the SNAP test. After the addition of this sample into nine parts of commercial milk reflecting the situation of most of the dairy farms, results were negative for both tests.

Agreement between tests

Results of the Cohen's κ test are presented in table 4. As expected Delvotest completely disagreed with the two

Table 1 Assessment of residues of ceftiofur and its metabolites in milk samples of six dairy cows dosed with 1 ml/50 kgBW Curacef Duo (Virbac, Mexico) for five consecutive days, according to the test Delvotest SP NT

	Delvotest SP NT (LDC [_{ceftiofur and DFC}]=80 ppb)											
	Cow #1 (low yielding)		Cow #2 (low yielding)		Cow #3 (high yielding)		Cow #4 (low yielding)		Cow #5 (high yielding)		Cow #6 (high yielding)	
	No	Dilution	No	Dilution	No	Dilution	No	Dilution	No	Dilution	No	Dilution
	1:4	1:10	1:4	1:10	1:4	1:10	1:4	1:10	1:4	1:10	1:4	1:10
T_{baseline}	–	–	–	–	–	–	–	–	–	–	–	–
T_0	–	–	–	–	–	–	–	–	d(–)	–	–	–
T_{12}	–	–	–	–	–	–	–	–	–	–	–	–
T_{24}	–	–	–	–	–	–	–	–	–	–	–	–

Samples were collected from the fifth day (T_0 , last injection) and twice a day during four days. Only results of the first 24 hours are displayed (0, 12 and 24 hours). Samples with positive results were diluted to 1:4 and 1:10 and tested again.
+, positive result; –, negative result; d(+, –, d), doubtful result confirmed either +, – or doubtful.
DFC, desturoy/ceftiofur.

Table 2 Assessment of residues of ceftiofur and its metabolites in milk samples of six dairy cows dosed with 1 ml/50 kgBW CURACEF DUO (Virbac, Mexico) for five consecutive days, according to the test ROSA MRL Beta-Lactam Test

ROSA MRL Beta-Lactam Test (LDC _[ceftiofur and DFC] = 10–20 ppb)												
	Cow #1 (low yielding)		Cow #2 (low yielding)		Cow #3 (high yielding)		Cow #4 (low yielding)		Cow #5 (high yielding)		Cow #6 (high yielding)	
	No	Dilution	No	Dilution	No	Dilution	No	Dilution	No	Dilution	No	Dilution
	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10
T _{baseline}	–	–	–	–	–	–	–	–	–	–	–	–
T ₀	d(+)	–	–	–	+	–	d(+)	–	+	d	–	–
T ₁₂	d(+)	–	+	–	+	–	+	–	–	–	+	–
T ₂₄	–	–	–	–	–	–	d(d)	–	–	–	–	–

Samples were collected from the fifth (T₀, last injection) and twice a day during four days. Only results of the first 24 hour are displayed (0, 12 and 24 hours). Samples with positive results were diluted to 1:4 and 1:10 and tested again.

+, positive result; –, negative result; d(+,–,d): doubtful result confirmed either +, – or doubtful.
DFC, desfurioceftiofur.

Table 3 Assessment of residues of ceftiofur and its metabolites in milk samples of six dairy cows dosed with 1 ml/50 kgBW Curacef Duo (Virbac, Mexico) for five consecutive days according to the test SNAP Beta-Lactam ST Plus

SNAP Beta-Lactam ST Plus (LDC _[DFC] = 30 ppb)												
	Cow #1 (low yielding)		Cow #2 (low yielding)		Cow #3 (high yielding)		Cow #4 (low yielding)		Cow #5 (high yielding)		Cow #6 (high yielding)	
	No	Dilution	No	Dilution	No	Dilution	No	Dilution	No	Dilution	No	Dilution
	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10	dilution 1:4	1:10
T _{baseline}	–	–	–	–	–	–	–	–	–	–	–	–
T ₀	d(+)	–	d(d)	–	+	–	–	–	+	+	–	–
T ₁₂	d(d)	–	+	–	+	–	d(+)	–	–	–	–	–
T ₂₄	–	–	–	–	–	–	–	–	–	–	–	–

Samples were collected from the fifth day (T₀, last injection) and twice a day during four days. Only results of the first 24 hours are displayed (0, 12 and 24 hours). Samples with positive results were diluted to 1:4 and 1:10 and tested again.

+, positive result; –, negative result; d(+,–,d), doubtful result confirmed either +, – or doubtful.
DFC, desfurioceftiofur.

Table 4 Between test agreement

	Test name	
	ROSA MRL Beta-Lactam Test	Snap betalactam
Delvotest ST NP	0.00 (0.38)	0.00 (0.36)
ROSA MRL Beta-Lactam	/	0.61 (0.14)

Cumulated results of the first four milking from the last treatment (T_0 , T_{12} , T_{24} and T_{36}). Cohen's κ coefficient (sd).

other tests ($\kappa=0.00$). A substantial agreement ($\kappa=0.61$) was found between the SNAP test and the ROSA test for the first four milking.

DISCUSSION

Studies conducted for nearly 30 years have imposed the idea that intramuscular injections of ceftiofur in the standard dose of 500 mg/cow always lead to concentrations in tissues and milk below detectable limits.¹² In this research, we found that milk produced by individual cows dosed with 1 mg/kgBW ceftiofur HCl for five consecutive days can occasionally display positive or doubtful results to some of the screening tests commonly used in the dairy industry. The risk of a positive result was clearly increasing as the estimated lowest detected concentrations (LDCs) at a specific detection capability level ($CC\beta$) decreased. None of the cows was detected positive with Delvotest, whereas all of them were found positive on T_0 or T_{12} , with the ROSA test or the SNAP test.

Positive results to screening test might reveal the presence of tiny amounts of active ceftiofur. Consequences of these small amounts of drug have been considered already in the calculation of the ADI. Moreover, the ceftiofur ADI takes in account the potential impact of ceftiofur and DFC on typical bacteria (*Escherichia coli*, *Lactobacillus* subspecies and *Clostridium* subspecies) of the human gut flora.⁶ Concentrations of ceftiofur/DFC in milk of 20–50 $\mu\text{g}/\text{kg}$ represent 0.02–0.05 $\mu\text{g}/\text{ml}$, whereas MIC_{50} of those typical bacteria are close to 2.0 $\mu\text{g}/\text{ml}$. Indeed exposure of bacterial species to subtherapeutic dosages of antibiotics must be avoided, but in this instance, residual concentrations are far lower than the therapeutic concentration, and ceftiofur will probably have no activity against bacteria.

In the validation study of Delvotest,¹³ the estimated LDC for ceftiofur was 100 $\mu\text{g}/\text{kg}$ for the ampoule format of the test. We failed to find information about the detection capability of DFC by the Delvotest; however, other researches have reported that Delvotest T, a similar *B. stearothermophilus*-based test is not able to detect the parent metabolite DFC.¹⁴ Moreover, in a recent technical bulletin, the manufacturer stated that the detection capability 5% ($CC\beta$, where $\beta=5\%$) of ceftiofur is 20 $\mu\text{g}/\text{kg}$, and the CC5 of both ceftiofur and its metabolites is approximately four times this level¹⁵ in normal milk. In our research, since Delvotest testing yielded only negative results, it can be reasonably

stated that the residual concentration of ceftiofur and its metabolites is always lower than the CC5 and very likely to be lower than the EU-MRL. Others came to the same conclusion with a similar ceftiofur HCl-based injectable medicine and the Delvotest (unpublished study report¹⁶), when applied to normal milk.

CC5 of the two receptor assays are not published. According to the SNAP test manufacturer's website, the LDC of ceftiofur is 9 $\mu\text{g}/\text{kg}$, whereas another manufacturer's sponsored publication¹⁷ reports 12 $\mu\text{g}/\text{kg}$ and 50–80 $\mu\text{g}/\text{kg}$ for ceftiofur and the parent metabolite (DFC), respectively. Slightly different figures were reported by independent researchers,¹⁸ with 25% and 70% of the EU MRL (25 $\mu\text{g}/\text{kg}$ and 70 $\mu\text{g}/\text{kg}$) for the ceftiofur and the DFC, respectively. With regard to the ROSA test manufacturer's online documentation that is inconsistent, the LDCs of ceftiofur and DFC together range from 10–20 $\mu\text{g}/\text{kg}$ to 30–60 $\mu\text{g}/\text{kg}$. Independent publications tended to fine tune these estimations to 10–20 $\mu\text{g}/\text{kg}$ for ceftiofur,¹⁹ or 6 $\mu\text{g}/\text{kg}$ and 10 $\mu\text{g}/\text{kg}$ for ceftiofur and DFC in fortified cow milk, respectively.²⁰ A more recent test based on the same technology (ROSA MRL BLTET, same manufacturer) was evaluated and allows the detection of ceftiofur at concentrations lower than 50 $\mu\text{g}/\text{kg}$ in ewe and goat milk.²¹

On T_0 and T_{12} , some cows showed positive results to the receptor assays followed with negative results when samples were diluted to reflect field testing conditions. Conversely, cows #2 and #4 showed negative or doubtful results at T_0 that turned doubtful or positive at T_{12} . This may indicate that ceftiofur concentrations in tested samples were very close to the LDCs of the test kits. Therefore, it can be assumed that ceftiofur and DFC concentration at T_0 , T_{12} and T_{24} for the cow #4, occasionally exceeded 10–20 $\mu\text{g}/\text{kg}$. This is aligned with results gained in the development phase of the test product with a High Pressure Liquid Chromatography-Mass Spectrometry (HPLC-MS) method where results were most of the time below the limit of quantification (41 $\mu\text{g}/\text{kg}$) with some outlining results (up to 70 $\mu\text{g}/\text{kg}$) and often below the limit of detection (16 $\mu\text{g}/\text{kg}$).²² In all cases, the sum of residual concentrations of ceftiofur and DFC was below the MRL of the drug.

Other studies reported a good agreement between microbial inhibitor screening tests and receptor tests,²³ whereas the Delvotest completely disagreed with the two other tests in our study.

Commission Decision 2002/657/EC²⁴ describes specificity as the ability of a method to distinguish between the analyte being measured and other substances. This characteristic is predominantly a function of the measuring technique described but can vary according to class of compound or matrix. Raw specificity of rapid screening test is necessarily low since they must be able to detect a wide range of substances. However, at the antibiotic class level, the specificity is generally high. Receptor tests specifically designed for the detection of the β -lactam ring accurately detect this structure.

The detection capability ($CC\beta$) and the decision limit ($CC\alpha$) are two important performance characteristics of confirmatory methods for banned substances. The $CC\beta$ has been defined in Decision 2002/657/EC as the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β . For rapid screening tests, these characteristics are not clearly indicated by the manufacturers, and regardless to the statistical significance, the LDC looks an appropriate threshold for the interpretation of the results.

Rapid screening tests have been designed to maximise the detection of inhibitory contaminants in raw milk at the dairy plant level. Test kits that allow the detection of minute quantities of prohibited substances are tools of risk management. It is certainly more desirable to discard thousand litres of potentially contaminated milk than to process tens of thousands of litres of contaminated milk by ignorance. Therefore, the key statistical quality of such a screening test is its predictive negative value (PNV) or its ability to tell that a sample is truly negative with a tiny probability error. To some extent, the PNV is in direct proportion of the specificity and the actual proportion of tank milk free of contaminants which is high in developed dairy countries. A recent publication reports that only 0.011% of tankers contained violative levels of β -lactams in the USA,²⁵ and the same situation is observed in France with only 575 tanks found positive to inhibitors in 2015 on 6 million tested.²⁶ Conversely, the predictive positive value (PPV) which, to some extent, combines the $CC\beta$ and the actual prevalence of contamination, is poor and false positive results are numerous. The larger the gap between LDCs and MRLs, the lower the PPV. Eventually, kit manufacturers have contended that the term false positive is incorrect and should be replaced by the term false violative or non-actionable positive result.²⁷ Positive results must be confirmed by another test based on another technology. Therefore, screening tests are particularly more reliable for commingled milk testing than for individual cow testing.

Many rapid screening tests do not specify the type of milk sample that must be collected for the analysis.²⁸ Whereas mastitis is the most common health-related condition of the dairy cow, the main cause of use of antibiotic treatment and a condition after which the milk from recovering cows is likely to be tested, several components in mastitic milk interfere with various antibiotic residue screening tests. These components include somatic cells, lactoferrin, lysozyme, microbes and free fatty acids, and they may hamper outcomes of microbial inhibitor tests.²⁹ Depending on the analytical principle of the screening test, these milk components may have a major impact on the outcome of the test, especially because milk is generally assayed shortly after the clinical outbreak, when the milk may still contain these components in relatively high concentrations. It is not necessary to remind the reader that drug approval process is almost exclusively based on studies carried out in healthy animals. Compared

with most other cephalosporins, the long excretion half-life of ceftiofur could potentially results in a cumulative effect. In two separate studies,⁶ doses of 2.2 mg/kgBW/day were administered to dairy cows for five consecutive days; concentrations in milk of ceftiofur metabolites were close (71 μ g/kg) to, and possibly slightly over (115 μ g/kg), the MRL, 12 hours and 10 hours, respectively, after the last dose. This potentially explains why some positive reactions have been observed at T_0 and T_{12} in our study.

Mastitis in dairy cows can also alter plasma PK of ceftiofur. It has long been shown that intramuscular ceftiofur does not achieve effective concentrations against mastitis pathogens in the normal or inflamed mammary gland.^{12,30} Despite the ceftiofur is well known to be used in dairy cattle, there is a paucity of data comparing PK parameters of the drug between healthy and diseased cows.³¹ In a somewhat old study,³⁰ the authors found that cows with induced mastitis and dosed with 3 mg/kgBW ceftiofur sodium had a detectable antimicrobial activity (>200 μ g/kg ceftiofur) in milk from 8 hours to 21 hours after the first injection and 26–31 hours for uninfected ones. They noted that concentration of ceftiofur in serum was higher at seven hours after each dose in non-infected cows, and they suggested that clearance in infected animals in more rapid. Recently (2016), Gorden and others,³¹ modelled serum kinetics of ceftiofur HCl in cows with severe endotoxic mastitis dosed with 2.2 mg/kgBW. They suggested that ceftiofur has a shorter plasma half-life in diseased animals, as well as an initially higher peak concentration, a higher volume of distribution and drug clearance rates. The authors suggested that altered PK parameters may contribute to an increased risk for the development of a violative residue in meat. Assessment of a more rapid and intense elimination of ceftiofur in mastitis cows, through natural excretory routes, needs further investigation. Nevertheless, it is unlikely that the very low excretion rate of Ceftiofur in milk will be significantly increased.

To complete this discussion, a simple calculation can support the previous conclusions. Assuming an excretion rate of ceftiofur HCl of 0.15% into the milk,⁶ a single daily injection of 600 mg to an adult dairy cow (1 mg/kgBW) would result in a maximum amount of 0.9 mg or 900 μ g of the antibiotic in the milk. Therefore, only 9 litres of milk could be theoretically contaminated at the MRL level which is lower than the daily milk production of low yielding cows. Consequently, contamination of large volume of milk by ceftiofur HCl is barely not possible, when used in an appropriate manner. Positive outcome to screening tests of bulk milk from a tank of tens of thousands litres can never be assigned to a 50 mg/ml ceftiofur HCl-based injectable medicine when used according to label instructions.

CONCLUSION

On occasion, individual cow's milk may give positive results when tested with two commercially available immunoreceptor screening tests, within the first 24 hours after

the end of the treatment. The classical microbiological screening test always yields negative results, suggesting that the residual concentration of ceftiofur and its residues in milk is very unlikely to exceed 80 µg/kg. Moreover, since dairy herds are not made up of one but several cows, careful injection of the tested ceftiofur HCl-based product to one cow is unlikely to result in positive outcomes to screening tests for commercial herds larger than nine animals. In dairy cows dosed with a combination of ceftiofur HCl/ketoprofen 50/150 mg/ml in compliance with the summary of the test product characteristics, residual concentration of ceftiofur in the milk should never exceed the maximum residue level (100 µg/kg).

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