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CORRELATION BETWEEN PLASMA CONCENTRATION OF A NEW CEFTIOFUR FORMULATION AND MINIMUM INHIBITORY CONCENTRATION VALUES OF ZOOTECHNICAL INTEREST MICROORGANISMS IN CATTLE AND PIGS.

CORRELAÇÃO ENTRE CONCENTRAÇÃO PLASMÁTICA UMA NOVA FORMULAÇÃO DE CEFTIOFUR E OS VALORES DE CONCENTRAÇÃO MÍNIMOS DE INIBIÇÃO DE CRESCIMENTO DE MICRORGANISMOS DE INTERESSE ZOOTÉCNICO EM BOVINOS E SUÍNOS

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SUMMARY

Ceftiofur is a third-generation cephalosporin widely used in cattle and swine farming to treat respiratory and reproductive conditions. The veterinary pharma industry consistently develops new formulations with this molecule to bring highly effective products to market with short withdrawal periods, ensuring consumer safety for products derived from treated animals. The study aimed to evaluate the plasma concentration profile of Vilocci, a new formulation of ceftiofur, in comparison to the minimum inhibitory concentrations (MIC) of various pathogens commonly found in bovine and swine farming. Ten animals from each species were used, and a single dose of 1 mg/kg was given to bovines and 5 mg/kg to swine. Blood samples were collected at 12 time points to construct the pharmacokinetic profile. The minimum inhibitory concentration (MIC) of growth for the pathogens of interest isolated from the target species was determined and the effectiveness of the for; mulation was assessed for each one.

KEY-WORDS: Antimicrobial. Cattle. Minimal inhibitory concentration. Pharmacokinetics. Swine

RESUMO

O ceftiofur é uma cefalosporina de terceira geração amplamente utilizado na bovinocultura e suinocultura para o tratamento de afecções respiratórias e reprodutivas. De forma recorrente, a indústria desenvolve novas formulações com esta molécula no intuito de trazer ao mercado produtos com alta eficácia e com curtos períodos de carência, promovendo segurança alimentar ao consumidor. Este trabalho avaliou a curva plasmática do produto Vilocci, a base de ceftiofur, frente a concentração inibitória mínima (CIM) de diversos patógenos presentes na rotina da bovinocultura e suinocultura. Utilizou-se 10 animais de cada espécie, e aplicada a dose de 1 mg/kg para os bovinos e 5 mg/kg para os suínos, ambos em dose única. Os animais tiveram seu sangue coletado seriadamente em 12 momentos a fim de montar a curva farmacocinética. Determinou-se a concentração inibitória mínima de crescimento (CIM) dos patógenos de interesse isolados na espécie alvo e verificou-se a eficácia da formulação frente a cada um deles.

PALAVRAS-CHAVE: Antimicrobiano. Bovinos. Concentração inibitória mínima. Farmacocinética. Suínos.

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INTRODUCTION

Ceftiofur, a third-generation cephalosporin, is effective against Gram-positive and Gram-negative bacteria, including beta-lactamase-producing strains (GORDEN, 2017).

It is used for treating respiratory and reproductive diseases in pigs, at the dose of 3 to 5 mg/kg intramuscularly (PAPICH, 2016), is effective in controlling colibacillosis in piglets, reducing weight loss, mortality and diarrhea (HIBBARD et al., 2003) and it is also a therapeutic option for metritis in cows, showing similar efficacy ampicillin (LIMA et al., 2014).

Researchers compared the efficacy of ceftiofur and enrofloxacin for the treatment of undifferentiated bovine respiratory disease. Both treatments were effective (ABUTARBUSH et al., 2012).

Ceftiofur is recommended for treating diarrhea in calves caused by *Escherichia coli* with systemic involvement (CONSTABLE, 2004) and doses of 5 mg/kg of ceftiofur led to a reduction in pathogen release in feces and improvement in appetite, diarrhea and lethargy in calves with *Salmonella* Typhimurium-induced diarrhea (FECTEAU et al., 2003).

In swine, ceftiofur is rapidly absorbed after parenteral administration and quickly metabolized into desfuroylceftiofur. The maximum concentration of 23-27 ug/mL was reached after 2 hours of administration, with an observed half-life of 19 hours (PAPICH, 2016).

In cattle, the intramuscular administration of 1 mg/kg of ceftiofur reached a peak concentration of 4,34 ug/mL in 2,4 hours after administration, with a half-life of 10 hours (EMEA, 1999).

Both the base molecule and its active metabolite have high plasma protein affinity, ensuring safe concentrations above therapeutic levels in a single dose, with 70% bioavailability (BROWN et al., 2010).

Cephalosporins, including ceftiofur, exhibit time-dependent action, relying on plasma concentrations exceeding the minimum inhibitory concentration of the target pathogen for therapeutic efficacy (SPINOSA et al., 2017). This study aims to compare 24-hour plasma concentrations after a single Vilocci application in pigs and cattle, assessing its efficacy against bacteria causing diseases in both species.

MATERIAL AND MÉTHODS

Ten pigs (5 males and 5 females) aged 60 to 80 days and weighing between 34 and 60 kilograms were used. Regarding cattle, ten adult bovines (5 males and 5 females), aged between 18 and 24 months, with a body weight ranging from 150 to 250 kilograms, were included.

The animals were selected for the study after their health was certified by a veterinarian, with normal values in hematological and biochemical parameters. During the study, the pigs were kept in collective concrete pens equipped with nipple drinkers and troughs, and they were fed with species-specific feed. The cattle, on the other hand, remained throughout the experimental period in a collective concrete paddock, with water available at will, and were fed twice a day with commercial cattle feed.

The animals received Vilocci (Ceftiofur hydrochloride 50mg/mL – UCBVET Saúde Animal) in different doses for each species, 5mg/kg of ceftiofur in swine and 1mg/kg of ceftiofur in bovines, with the dose applied intramuscularly in the region of the semitendinosus/semimembranosus muscles (gluteal).

Twelve blood samples were collected from each animal, with the first sample taken before treatment (0h) and the subsequent ones after the administration of the medication at the following time points: 0.5h, 1h, 1.5h, 2h, 3h, 5h, 7h, 9h, 10h, 12h, and 24h. The collections were performed in a restraint chute, using disposable needles and syringes, through jugular vein venipuncture. Blood was stored in appropriately labeled EDTA tubes. Subsequently, the samples were centrifuged at 5000rpm/10min, separated, and kept at -20°C until sent to the analytical laboratory.

The samples were analyzed using a validated method for quantifying the active metabolite of ceftiofur, and desfuroylceftiofur, in swine plasma at the Labfor laboratory in Campinas, SP.

The ceftiofur MIC of the selected bacteria was determined by preparing serial dilutions of the compound at decreasing concentrations (256 µg/mL to 0.125 µg/mL) in a specific culture medium (Adjusted Cation Muller-Hinton Broth). Field isolates were challenged with various concentrations of the tested compound. The test was conducted on flat-bottom 96-well plates. The MIC was based on the absence of cloudiness at the lowest concentration of the compound on microbial cultures. The interpretation of results (utilizing breakpoints) followed the guidelines of the Clinical Laboratory Standards Institute (CLSI). Microbiological analyses were carried out by the Nowavet Microbiology Laboratory.

The selected bacteria for the swine study included those commonly found in pig farming causing respiratory, gastrointestinal, joint, and dermatological disorders. Strains of *Salmonella enterica Choleraesuis, Pasteurella multocida A, Bordetella bronchiseptica, Haemophilus parasuis, Actinobacillus pleuropneumoniae, Salmonella* Typhimurium, *Clostridium perfringens, Clostridium difficile, Streptococcus suis serotype 1, Streptococcus suis serotype 2, Escherichia coli β hemolytic K88+, Staphylococcus aureus,* and *Staphylococcus hyicus* were used.

For bovines, the selection of the sample pool followed the same principle, including bacteria commonly found in bovine health challenges. Strains of *Pasteurella multocida* isolated from bovine arthritis, *Escherichia coli* and *Corynebacterium bovis* isolated from cows with mastitis, *Salmonella* Dublin in bovine milk, *Salmonella* Typhimurium from calf diarrhea, *Mannheimia haemolytica*, and *Fusobacterium necrophorum* from bovine lungs, and *Trueperella pyogenes* from bovine skin suppuration were chosen.

The analyses were performed with the assistance of the PKF complement in the Excel system (AUC, Tmax, Cmax). R 4.3.1 for Windows was used in the creation of figures.

RESULTS AND DISCUSSION

The obtained MIC values with isolates of the selected bacteria are presented in Table 1. All bacterial isolates used in determining the MIC are of national origin and from the target species of the study, aiming to mimic the epidemiology of these pathogens in national animal production.

Table 1 – Ceftofur MIC for the tested agents isolated from swine or bovines.

Agent	**MIC (ug/mL)**	**Host species**	I**solate**
Actinobacillus pleuropneumoniae	0,25	Swine	Swine lung
Bordetella bronchiseptica	0,50	Swine	Swine lung
Clostridium difficile	1,00	Swine	Swine intestine
Clostridium perfringens	0,50	Swine	Glässer disease
Escherichia coli β hemolítica K88+	0,50	Swine	Swine intestine
Haemophilus parasuis	0,25	Swine	Swine intestine
Pasteurella multocida A	0,125	Swine	Swine feces
Salmonella enterica Choleraesuis	0,50	Swine	Swine intestine
Salmonella enterica Typhimurium	2,00	Swine	Swine endocarditis
Staphylococcus aureus	0,25	Swine	Pigglet artristis
Staphylococcus hyicus	0,25	Swine	Swine feces
Streptococcus suis sorotipo 1	0,50	Swine	Swine meningitis
Streptococcus suis sorotipo 2	0,25	Swine	Pyogranulomatous dermatitis
Pasteurella multocida	0,5	Bovine	Bovine arthritis
Escherichia coli	0,5	Bovine	Mastitis
Fusobacterium necrophorum	0,5	Bovine	Bovine lungs
Corynebacterium bovis	0,5	Bovine	Mastitis
Salmonella typhimurium	0,25	Bovine	Calf diarrhea
Trueperella pyogenes	0,25	Bovine	Skin suppuration
Salmonella dublin	0,125	Bovine	Bovine milk
Mannheimia haemolytica	0,125	Bovine	Bovine lungs

It is observed that the highest MIC was 2 µg/mL of Ceftiofur, exhibited by *Salmonella enterica* Typhimurium, while the lowest MIC was 0.125 µg/mL, demonstrated by isolates of *Pasteurella multocida* A, *Salmonella* Dublin, and *Mannheimia haemolytica*. The remaining bacteria showed MIC values ranging between 1 and 0.25.

Table 2 presents the mean of maximum concentrations (Cmax) at each time point, along with standard deviations and the time to reach maximum concentration (Tmax). The maximum concentration achieved is consistent with that found by Brown (BROWN et al., 1991) for the same dose in pigs, and the same is observed for the time to reach maximum concentration. In

cattle, the maximum concentration of 4.03 µg/mL obtained was close to that reported in calves at the same dose by EMEA (EMEA, 1999), and the time to reach the maximum concentration was in line with the findings of Wang (WANG, 2017).

Table 2 - Mean of maximum plasma concentrations (Cmax) for each evaluated time point followed by their respective standard deviations for swine and bovine species

Swine			**Bovine**	
Time (hours)	**Cmax (ug/L)**	**DP (ug/mL)**	**Cmax (ug/mL)**	**DP (ug/mL)**
0	0	0	0	0
0,5	10,88	4,37	2,45	0,96
1	21,58	9,31	3,11	0,56
1,5	23,13	8,57	3,60	0,66
2	20,2	5,56	3,78	0,68
3	17,89	5,40	3,42	0,78
5	12,39	3,69	2,55	0,55
7	11,60	3,15	2,03	0,48
9	7,44	3,26	1,50	0,39
10	6,37	2,32	1,20	0,30
12	5,28	2,02	0,87	0,30
24	3,09	1,47	0,55	0,33
Cmax* (µg/mL)	27,2	8,166	4,07	0,63
Tmax* (h)	1,65	0,78	1,75	0,68
AUC_{0-t} (µg.h/mL)	194,08	68,4995	35,27	10,09

*Cmax: Maximum plasma concentration
*Tmax: Time to maximum plasma concentration
*AUC_{0-t}: Area under curve from zero to the last timepoint.

Ceftiofur, like other third-generation cephalosporins, has a longer half-life, allowing for shorter therapeutic regimens. Due to its time-dependent activity, its effectiveness is ensured when plasma concentration remains above the minimum inhibitory concentration for at least 50% of the dosing interval (PAPICH, 2014).

Figures 1 and 2 depict the measured plasma concentrations over 24 hours and the MIC obtained from the bacteria listed for study in each evaluated animal species.

Figure 1. Ceftiofur plasma concentration (µg/mL) after a single 1 mg/kg dose in cattle over time, along with the MIC of the studied bacteria.

It can be observed a different scale between the plasma concentrations of ceftiofur obtained in pigs and cattle. This difference is directly related to the administered dose, as pigs received 5 times the dose of cattle, as per the specified dose for each species (PAPICH, 2014; EMEA, 2008).

In both figures, it is evident that the plasma concentration remains above the MIC of all studied pathogens (maximum MIC for swine pathogens = 2 µg of ceftiofur/mL, maximum MIC for bovine pathogens = 0.5 µg of ceftiofur/mL) throughout the entire evaluated period.

The Ministry of Agriculture and Livestock (MAPA) conditions the effectiveness of cephalosporins based on the "time of active plasma concentration above the MIC for more than 60% of the evaluated period" (T>CIM>60%). Therefore, the injectable suspension evaluated, based on ceftiofur hydrochloride, demonstrated efficacy against all pathogens isolated from swine and cattle.

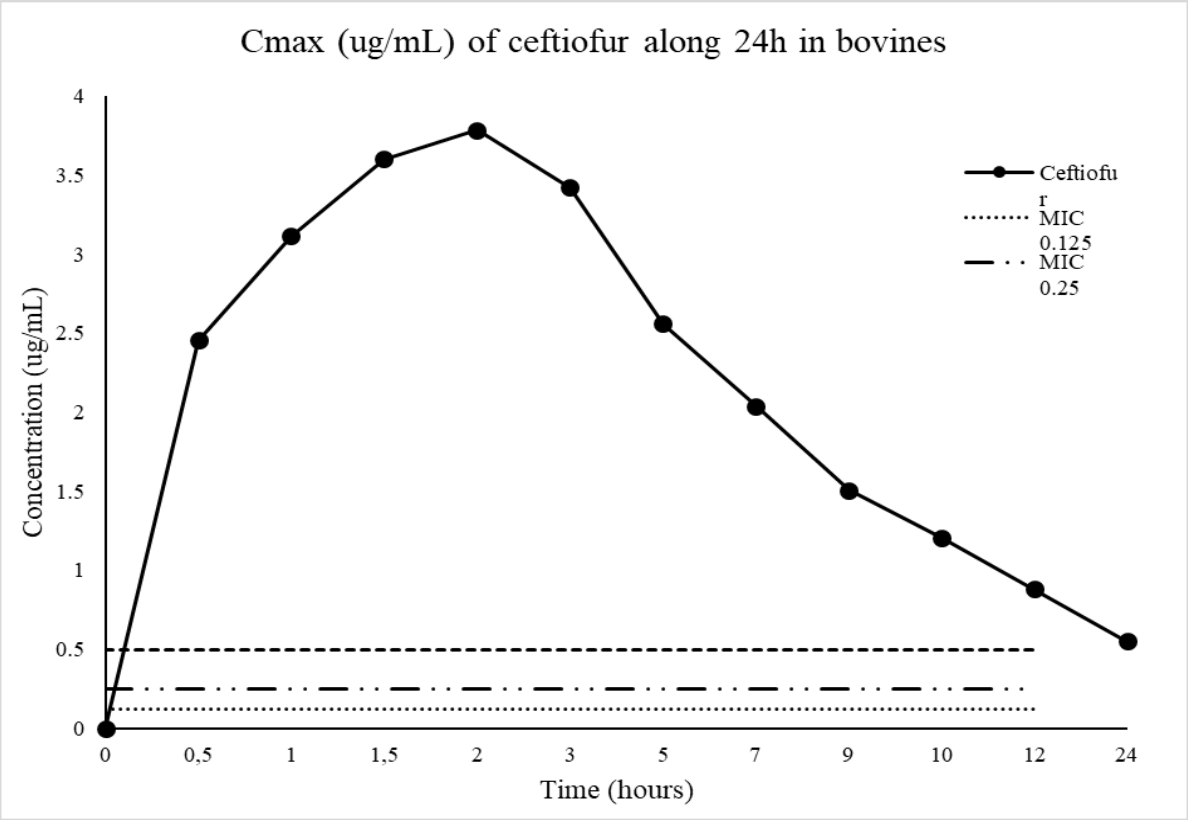

Figure 1 - Plasma concentration of ceftiofur (ug/mL) after application of 1 mg/kg in a single dose in bovines over time and the MIC of the evaluated bacteria

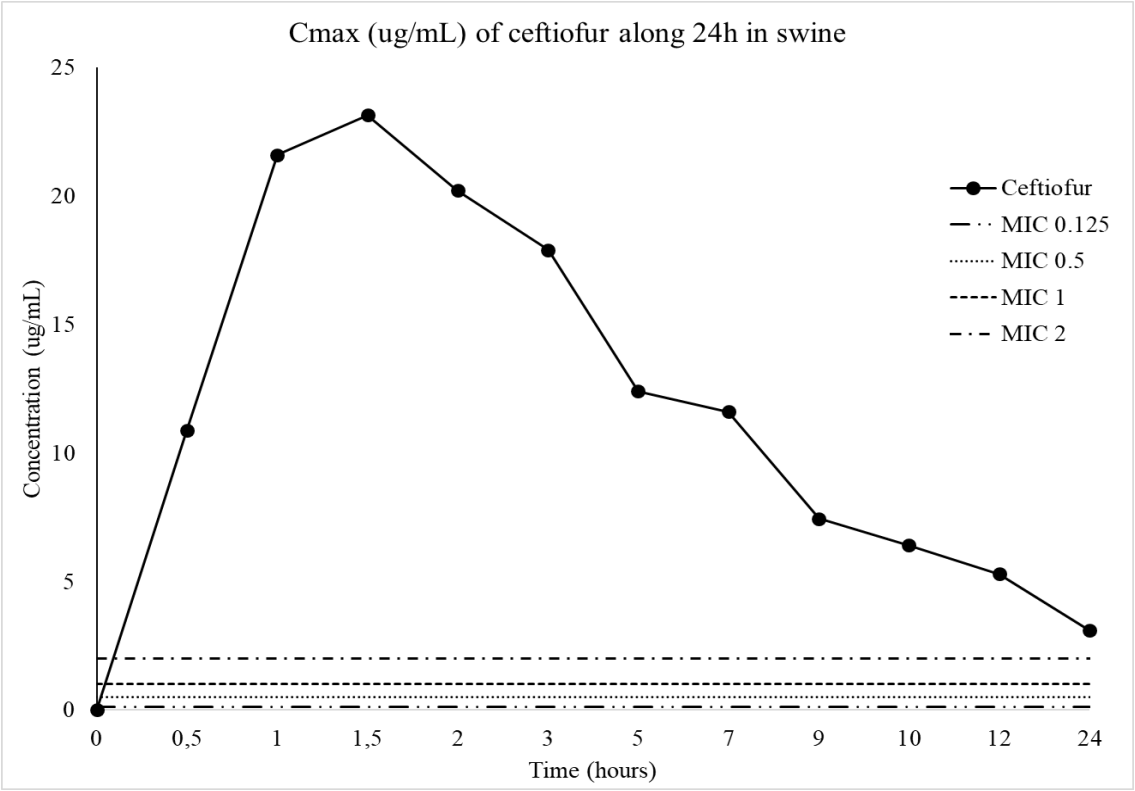

Figure 2 - Plasma concentration of ceftiofur (ug/mL) after application of 5 mg/kg in a single dose in swine over time and the MIC of the evaluated bacteria.

CONCLUSION

Vilocci, a formulation of ceftiofur hydrochloride, tested at a dose of 5 mg/kg administered intramuscularly in pigs, was effective against *Salmonella enterica* Choleraesuis, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, *Salmonella* Typhimurium, *Clostridium perfringens*, *Clostridium difficile*, *Streptococcus suis* serotype 1, *Streptococcus suis* serotype 2, *Escherichia coli* β hemolytic K88+, *Staphylococcus aureus*, and *Staphylococcus hyicus*.

In cattle, the dose of 1 mg/kg administered intramuscularly was effective against *Pasteurella multocida*, *Escherichia coli*, *Fusobacterium necrophorum*, *Corynebacterium bovis*, *Salmonella* Typhimurium, *Trueperella pyogenes* (*Arcanobacterium pyogenes*), *Salmonella* Dublin, and *Mannheimia haemolytica*.

The substantial difference between the MIC values obtained from strains isolated in the field and the plasma concentration values obtained and maintained during the evaluated period, in addition to providing 100% efficacy against such pathogens, reduces the possibility of selecting resistant strains. The tested formulation, therefore, proves to be an important tool for sanitary control in pig farming, demonstrating effectiveness against pathogens that act in various stages of production, and also in cattle farming, targeting microorganisms present in dairy production and beef cattle confinement.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The study was conducted in Coimbra, MG, and its execution occurred after approval from the Ethics Committee for the Use of Animals at NOWAVET Animal Health, under CEUA registration 01/2018

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