Is the suckling period and application pattern relevant for fluazuron efficacy against ticks in cows and their suckling calves?

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Abstract

Background: Fluazuron is a chitin synthesis inhibitor administered as a pour-on formulation in cattle for tick control. This study analyzes under endemic tick infestation, the incidence of the pour-on application pattern on the plasma levels of fluazuron in cows and calves in the lactation period of the beef cow. Three hundred and thirteen beef cows around parturition were treated with commercial pour-on formulation of fluazuron at a rate of 2.5 mg/kg of body weight. A total of 4 treatments were carried out with a dosing period set in a range of 32 to 45 days. At each administration time, the cows were grouped according to the pour-on administration pattern: long (~60 cm pour-on application surface) and short (~30 cm pour-on application surface). Fluazuron levels in cattle and calves plasma were determined before the third and fourth application for each subgroup (n=10) by HPLC-MS/MS. During the entire study cow–calf pairs were maintained under field conditions and qualitatively examined for tick infestation on the day of each treatment. Both treatment (long and short) schemes were designed to prevent the annual persistence of ticks.

Results: None of the groups presented ticks during the first 117 days of the study. There were no differences after 40 days post-treatment of the second application (30±5 ppb vs 28.5±12 ppb, P>0.05) and 45 days after the third application (147±55 ppb vs 140±46 ppb, P >0.05) between groups of cows treated with the long or short pour-on application, respectively. Plasma concentration of fluazuron at second and third application was increased (3.3 and 2.9 times, respectively) in calves under free suckling compared to cows. Nevertheless, both groups of cows (4.9 times) and calves (2.8 times) showed a significant increase in plasma concentration of fluazuron between times (P<0.05). In both groups, tick prevalence was 0% throughout the trial, except for day 77, which reached 1%.

Conclusions: The main conclusions of this study were the following: 1) Different administration patterns (long vs short) did not differ in plasma levels of fluazuron.; 2) Given that only the cattle were treated, and lactating calves presented higher plasma levels of fluazuron than cows, passage through milk appears to be relevant and possibly due to a cumulative effect and continuos drug intake.

Background

Tick infestations constitute one of the major problems in livestock production in Uruguay and South America (1, 2). The use of acaricides in Uruguay has been the main tool for tick control (3). In control programs of ticks, drugs administration requires a strategic dosage plan with established times according to the residuality of the drugs or depending if the establishment is in a tick control or eradication program(4). Dip acaricides used are organophosphates, pyrethroids and amidines. While injectable and pour-on applications are presented for macrocyclic lactones, fipronil and fluazuron (5). Resistance has been reported for all the aforementioned, except for fluazuron in which there is no practical *in-vitro* tests for its detection (6).

Fluazuron is responsible for inhibiting chitin synthesis on immature ticks, preventing them from achieving adult stages. Insect growth regulators, such as fluazuron, could be responsible for the long-term response given the delayed effect that this type of compounds have on insect´s life cycle (7), reducing the number of immature life stages of *Rhipicephalus microplus* (*R. microplus*).

The application of fluazuron pour-on formulation in the initial period of lactation of the cow, generates uncertainty about the levels reached in cattle or their lactating calves. Likewise, the persistence levels or the elimination rate of fluazuron in lactating cow beefs is not clear (8). Concentration levels due to digestive absorption in lactating calves are also not reported. Furthermore, differences in the practical application technique at the field level, using different empirical recommendations in the topical administration of the product, varying the extension on the application surface, but maintaining the dose, remains as an uncertainty.

Therefore, the efficacy and associated benefit of the fluazuron poured preparation as a tick control alternative in lactating cows needs to be documented. The objective of this study was to determine in endemic tick infestation the incidence of the application pattern of fluazuron pour-on on the plasma levels of this acaricide in cows and calves in the lactation period of the beef cow.

Results

The prevalence of ticks at the beginning of the experiment (0 day) was 1% (3/313), as expected by the epidemiological model described for Uruguay. In the following evaluations (32, 77 and 117 days), no animals with presence of ticks were identified, except three animals (in short group 0.7 [1/145] and in long group 1.3 [2/147], $P > 0.05$) and one calf at the time of the third application (77 day). The infestation rate was not different ($p > 0.05$) between the two application surfaces groups throughout the evaluation periods.

The individual levels of fluazuron in cattle determined prior to the third and fourth repeated application for each subgroup ($n = 10$) are presented in Fig. 1. There were no differences after 40 days post-treatment of the second application (30 ± 5 ppb vs 28.5 ± 12 ppb, $P > 0.05$) and 45 days after the third application (147 ± 55 ppb vs 140 ± 46 ppb, $P > 0.05$) between the groups of cows treated with the long or short pour-on application, respectively. In view that the cattle groups (long vs short) did not present statistical differences, it was considered as a global cattle group. Forty days after the second administration, the average concentration (± standard deviation) for global cattle group and calves were 29.3 ± 8.9^{a} ppb and 147 ± 43^{b} ppb ($P < 0.05$), respectively (Fig. 2). The application of the third repeated treatment and the determination of the concentrations 45 days later increased the average concentration reaching 143 ± 49^{a} ppb and 417 ± 87^{b} ppb ($P < 0.05$) in the treatments of global cattle and calf groups, respectively (Fig. 2). Plasma concentration of fluazuron prior to the third and fourth application increased 3.3 and 2.9 times (respectively) in calves under free suckling vs. cows (Fig. 2). Nevertheless, the global cattle group (4.9 times) and the calves (2.8 times) showed a significant increase in plasma concentration of fluazuron, between 77 at 117 days ($P < 0.05$) (Fig. 2).

Discussions

The bioavailability of a drug is directly dependent on the rate and degree of absorption of the drug at the site of administration. Factors that affect the absorption of the drug, including patterns for the pour-on application, will directly affect the bioavailability of the drug. Our results suggest that the drug dispersion pattern long vs short, does not represent a relevant variable when the correct dose is respected. The topical absorption of pour-on formulation can be explained through the Fick law of diffusion in which the drug molecule moves according to the concentration gradient from a higher drug concentration to a lower one until equilibrium is reached (9). Although in our study we doubled the absorption surface, we did not modify the dose, therefore the concentration was decreased per contact surface, which possibly determined that the passive diffusion of the drug was not significantly modified.

When redosing lipophilic drugs such as fluazuron at established times, not only the pharmacokinetic profile on the dam is affected, but also the plasma levels on lactating calves. After pour-on treatment at 1.5 mg/kg, the mean plasma levels remained quite stable between 9 and 35 days after treatment, ranging from 35 to 41 ppb and declined at about 7 ppb at 16 weeks (10). Using the same dose and formulation, but from a single administration and during a short period of time, Ferreira et al., (2019) reported lower plasma concentration values. In our study, the dosing interval was set at 4–6 weeks following the manufacturer recommendations and the governmental guidelines on tick control for Uruguay, which could explain the higher concentration levels of fluazuron found in cattle and calves. The Committee for Medical Products for Veterinary Use (11) issued a Summary report on fluazuron pharmacokinetic behavior, with fluazuron being excreted via cow's milk to calves, resulting in higher plasma and fat residue levels in calves compared to the ones in cows.

Accumulation is usually considered with regard to plasma, overlooking accumulation of the drug in tissues with poor blood perfusion (12), leaving the accumulation of drug concentration on lactating animals relegated when it comes to explaining whether accumulation occurs and the possible impacts of such process. According to (11), a steady state between absorption and elimination was observed for three to four weeks after treatment. When a single dose of fluazuron is administered topically to cattle, the depletion from plasma is slow, with an elimination half-life of 10.5 weeks. Therefore, multiple doses could lead to drug accumulation both in the cow and the calves. As reported by (11) multiple treatments with 12-week intervals did not lead to accumulation of fluazuron residues.

The greater accumulation of this acaricide in lactating calves in relation to cows and the increase between subsequent administrations, could be explained by the continuous intake via the digestive tract through milk. Different authors document the importance of licking in the topical administrations and the relative importance of the dermal and oral routes in the removal of drugs from the skin (13, 14). We ruled out the licking effect since at the time of application we made a quick visual inspection and determined a null licking behavior in the first hours after applying all treatments (Unpublished Data). As stated by (15), excretion of parent compounds and/or their metabolites from plasma into milk is a complex process related with physico-chemical properties and membrane interactions. Fat content is one of the main

factors that contribute to the concentration of hydrophobic drugs into milk (16, 17). Given the lipophilic characteristic of fluazuron, fat and milk residue levels are expected to be higher than the plasma levels.

In this study, the prevalence and burden of *R. microplus* found in the animals is consistent with the data provided by the conceptual epidemiological model described by (1) for Uruguay. Under ideal conditions of temperature and relative humidity (27°C and over 80% RH), the first generation of ticks takes place between August and October while the second generation appears from December to February. The tick populations parasitizing cattle are higher in relation to the first generation, with an average of 25 ticks per animal. Considering that the study was carried out in a farm with a history of presence of ticks in previous autumn months, and that the prevalence was lower than expected by the epidemiological model, we can consider that the concentration levels of fluazuron in the animals were sufficient to prevent the development of the parasitic cycle of the tick.

The rational use of drugs is crucial in food-producing animals because of the potential adverse effects of drugs that appear as residues in edible tissues (18–20). Milk excretion needs to be quantified in order to understand the pharmacokinetic changes that could occur during the suckling period and the impact on parasites control and drug residue levels, especially on parasites with high health impact such as *R. microplus*. Further studies are needed to quantify milk excretion of fluazuron under pour-on administration on lactating cows. Achieving a pharmacokinetic model for this excretion route is the first step on understanding the use of this chemical tool for tick control around parturition.

Conclusions

The main conclusions of this study were the following: 1) Different administration patterns (long vs short) did not differ in plasma levels of fluazuron; 2) Given that only cattle were treated, and lactating calves presented higher plasma levels of fluazuron than cows, passage through milk appears to be relevant and possibly due to a cumulative effect and continued drug intake.

Methods

Study location

The study was conducted from August to December 2019 in a farm located in Artigas, Uruguay, South America (30°06' South, 57°04'West). The farm has a history of tick and use of all approved drugs (Ivermectin, Fipronil, Amitraz or Fluazuron) for the control of *R. microplus*. Animals were not treated with acaricides in the 60 days previous to the fluazuron treatment. Tick infestations were present in a very low number of animals in the farm prior to the study. The prevalence was the expected for the period selected for the trial, which corresponds to the first generation of ticks within the epidemiological model of the country (1). Rainfall during 2019 (January to December) was 1440 mm and the minimum and maximum temperatures were 4.6°C (July) and 30.9°C (October), respectively.

Experimental design

Three hundred and thirteen beef cows (Hereford) around parturition were treated with commercial pour-on formulation of fluazuron (fluazuron 2.5%, pour-on, Acatak Pour-on®, Novartis, Brasil) at a rate of 2.5 mg/kg of body weight. A total of 4 treatments were carried out with a dosing period set in a range of 32 to 45 days. At the administration time two different patterns for the pour-on application were randomly tested and used as a grouping variable: *long* (~ 60 cm pour-on application surface, n = 163) and *short* (~ 30 cm pour-on application surface, n = 150). The calves remained free of antiparasitic treatment. Throughout the study, cow-calf pairs were kept in the same paddocks and under field conditions. Both treatment schemes were designed to prevent the annual persistence of ticks. A control group was not included because the farm was in a global tick control strategy.

Data recovery

Quantitative analysis of tick infestation:

All the cattle and calves in the study were qualitatively examined for tick infestation the day of each treatment (0, 32, 77 and 117 day). Each animal was examined for presence of ticks (adult engorged female ticks) (4.5–5 mm of diameter)) on the head, ears, neck, back, perineum and tail (21). A careful examination was carried out in 10 percent of the animals (randomly selected), which included the examination of the belly and legs. Presence of one or more ticks on an animal was recorded as a positive case.

Fluazuron Analytical procedures

Before the third and fourth application, blood was taken from 10 animals from each group of cows (*long* and *short*) and 10 calves randomly (using heparinized tubes).

Fluazuron concentration in plasma was determined by HPLCMS/MS, based on the procedure validated by our group obtaining acceptable accuracy according to the guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed from the European Commission (22). Matrix-matched calibration curves in the range from 5 to 1000 ppb (µg/kg) were established using least squares linear regression analysis and correlation coefficients (r = 0.99) and back calculated concentration residuals below 20%. A limit of quantification (LOQ) of 5 ppb was defined as the lowest measured concentration with relative standard deviation below 20% and an absolute recovery ≥ 70%. Concentration values below the LOQ were not considered in the analysis of experimental data.

Statistical analysis

The prevalence (%) of ticks was determined as the ratio of the number of existing cases during a certain period of time and the population at risk during that specified period (23). The plasma concentration data are reported as arithmetic mean ± SD. Differences between cattle groups (*long* or *short*) at each time were analyzed for statistical significance using the independent samples t test. Due to the fact that the long and short groups did not present statistical differences, they were considered a single group of cattle (global cattle group). The differences between calves and global cattle groups within and between times were analyzed for statistical significance using the independent samples t test.

Statistical analyses of differences in the prevalence rates between groups were performed with Fisher's exact test. A p value < 0.05 was considered statistically significant. The statistical analysis was performed using R Statistical Software (version 4.0.3 [2020-10-10]) (24).

List Of Abbreviations

HPLC-MS/MS = High Pressure Liquid Chromatography – Mass Chromatography / Mass Chromatography

Declarations

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. The present study was approved by the Comisión Ética en Uso de Animales (CEUA) of Facultad de Veterinaria-Universidad de la República, under protocol No. CEUA FVET-PI 506-1493294137. The study was carried out in compliance with the ARRIVE guidelines.

Informed consent was obtained from farm owners for including animals in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Authors do not have any competing interest to declare.

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Authors' contributions

GS conceived, designed, and supervised the project. DR and GS performed the experiment and analysed the data. AM, FP and LP determined fluazuron concentration. TS contributed on field experiments. GS, DR and LP wrote the manuscript, with contributions from SA. All authors reviewed the manuscript.

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References

1. Fiel C, Nari A. Enfermedades parasitarias de importancia clínica y productiva en rumiantes. : fundamentos epidemiológicos para su diagnóstico y control. Segunda. Montevideo: Hemisferio sur; 2013. 0–752.
2. Molento MB. Avaliação seletiva de bovinos para controle do Rhipicephalus microplus. Ars Veterinaria. 2020;36(1).
3. Miraballes C, Riet-Correa F. A review of the history of research and control of Rhipicephalus (Boophilus) microplus, babesiosis and anaplasmosis in Uruguay. Vol. 75, Experimental and Applied Acarology. 2018.
4. Cuore U, Acosta W, Bermúdez F, da Silva O, García I, Pérez Rama R, et al. Tratamiento generacional de la garrapata: Aplicación de una metodología en un manejo poblacional para la erradicación de Rhipicephalus (Boophilus) microplus resistentes a lactonas macrocíclicas. Veterinaria. 2015;51(197):44–52.
5. Cuore U, Solari M, Trelles A. Current status of resistance and first diagnostic of multiple resistance Rhipicephalus (Boophilus) microplus tick simultaneously resistant to five drugs in Uruguay. SMVU Veterinaria. 2017;53(205):13–9.
6. Reck J, Klafke GM, Webster A, Dall'Agnol B, Scheffer R, Souza UA, et al. First report of fluazuron resistance in Rhipicephalus microplus: A field tick population resistant to six classes of acaricides. Veterinary Parasitology. 2014;201(1–2):128–36.
7. Holdsworth PA, Vercruysse J, Rehbein S, Peter RJ, Letonja T, Green P. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of ectoparasiticides against biting lice, sucking lice and sheep keds on ruminants. Veterinary parasitology [Internet]. 2006 Feb 28 [cited 2016 Apr 18];136(1):45–54. Available from: http://www.sciencedirect.com/science/article/pii/S0304401705005510
8. Junquera P, Hosking B, Gameiro M, Macdonald A. Benzoylphenyl ureas as veterinary antiparasitics. An overview and outlook with emphasis on efficacy, usage and resistance. Parasite. 2019;26:26.
9. Neupane R, Boddu SHS, Renukuntla J, Babu RJ, Tiwari AK. Alternatives to biological skin in permeation studies: Current trends and possibilities. Vol. 12, Pharmaceutics. 2020.
10. Pronk MEJ, Schefferlie GJ. Fluazuron [Internet]. The forty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva; 1997 [cited 2021 Apr 5]. Available from:

http://www.inchem.org/documents/jecfa/jecmono/v39je09.htm

11. EMEA. Committe for Medical Products for Veterinary Use: Fluazuron Summary Report [Internet]. 2005. Available from: http://www.emea.eu.int

12. Brocks DR, Mehvar R. Rate and Extent of Drug Accumulation after Multiple Dosing Revisited. Clin Pharmacokinet. 2010;(7):421–38.

13. Laffont C, Bousquet-Mélou A, Bralet D, Alvinerie M, Fink-Gremmels J, Toutain P-L, et al. A pharmacokinetic model to document the actual disposition of topical ivermectin in cattle A pharmacokinetic model to document the actual disposition of topical ivermectin in cattle A pharmacokinetic model to document the actual disposition of topical ivermectin in cattle. Veterinary Research [Internet]. 2003;34(4):445–60. Available from: https://hal.archives-ouvertes.fr/hal-00902759

14. Toutain PL, Lees P. Integration and modelling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine. Journal of Veterinary Pharmacology and Therapeutics. 2004;27(6):467–77.

15. Shoop WL, Egerton JR, Eary CH, Haines HW, Michael BF, Mrozik H, et al. Eprinomectin: A Novel Avermectin for Use as a Topical Endectocide for Cattle. Vol. 26. 1996.

16. Begg E, Atkinson H, Duffull S. Prospective evaluation of a model for the prediction of milk:plasma drug concentrations from physicochemical characteristics. British Journal of Clinical Pharmacology. 1992;33(5).

17. Atkinson HC, Begg EJ. Relationship between human milk lipid–ultrafiltrate and octanol-water partition coefficients. Journal of Pharmaceutical Sciences. 1988;77(9).

18. Beyene T. Veterinary Science & Technology Veterinary Drug Residues in Food-animal Products : Its Risk Factors and Potential Effects on Public Health. 2016;7(1):1–7.

19. Gebeyehu DT. Review on Rational Use of Veterinary Antimicrobials and Anthelmintics. Austin Journal of Veterinary Science & Animal Husbandry. 2018;5(2).

20. Whittem T, Whittem JH, Constable PD. Modelling the concentration-time relationship in milk from cattle administered an intramammary drug. Journal of Veterinary Pharmacology and Therapeutics. 2012;35(5):460–71.

21. Holdsworth PA, Kemp D, Green P, Peter RJ, de Bruin C, Jonsson NN, et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of acaricides against ticks (Ixodidae) on ruminants. Veterinary Parasitology. 2006 Feb 28;136(1 SPEC. ISS.):29–43.

22. European Commission. European Union, 2002, COMMISSION DECISION 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities, L 221/8. Official Journal of the European Communities, L 221/8. 2002;29.

23. Thrusfield M. Veterinary Epidemiology. Equine Internal Medicine: Second Edition. 2004.

24. RStudio Team (2020). Integrated development for R. RStudio. 2020.

Figures

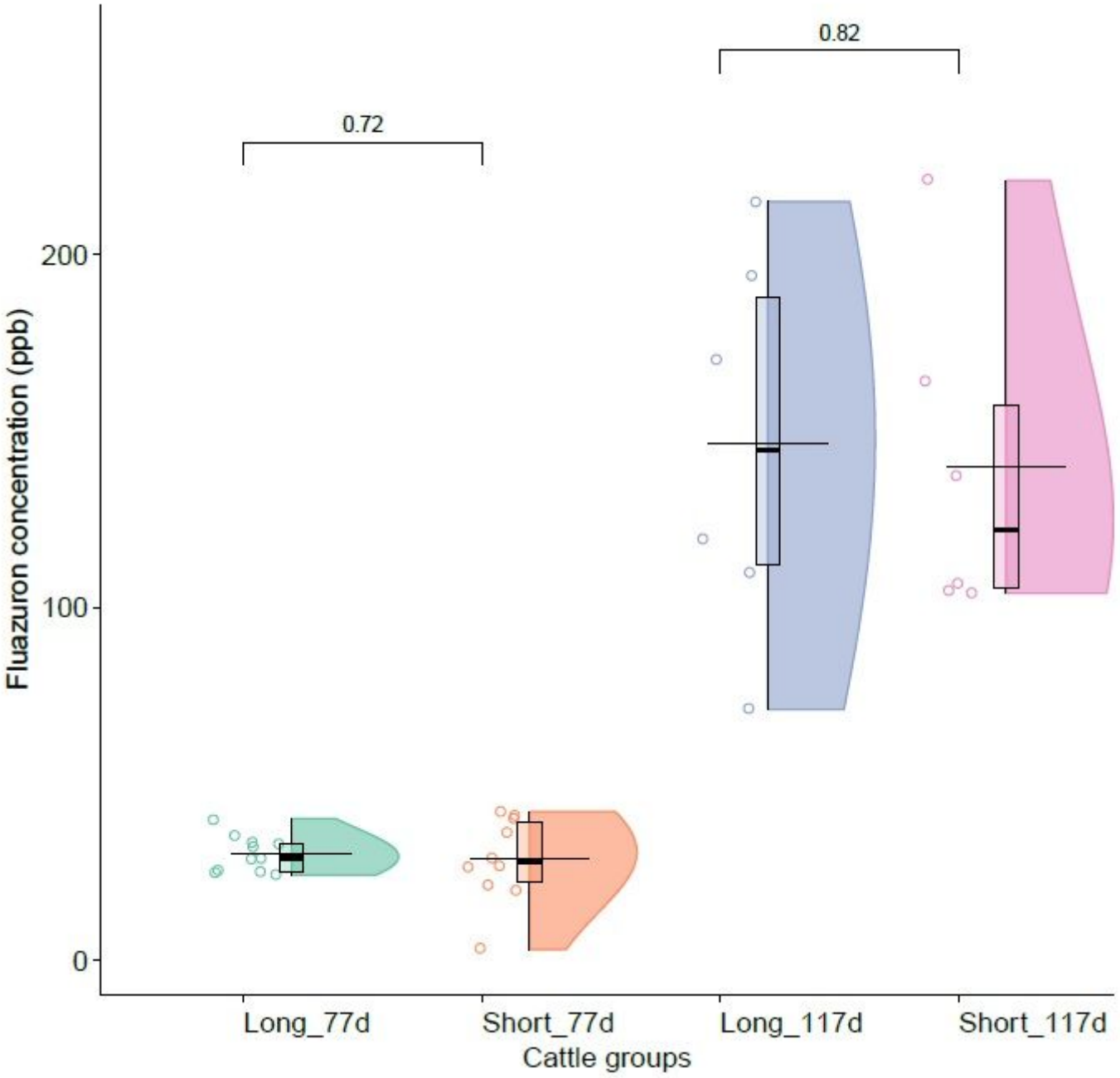

Figure 1

Fluazuron adult cattle plasma concentration (ppb) before dose repetition (77 and 117 days from first dose) of a fluazuron pour-on formulation (2.5 mg/kg, pour-on, 2.5%, Acatak®), following two different application schemes: short (30 cm) and long (60 cm). Long_77d = long application at 77 day; Short_77d = short application at 77 day; Long_117d = long application at 117 day; Short_117d = short application at 117 day. P Value Pairwise (T-test) indicates the significant difference between groups.

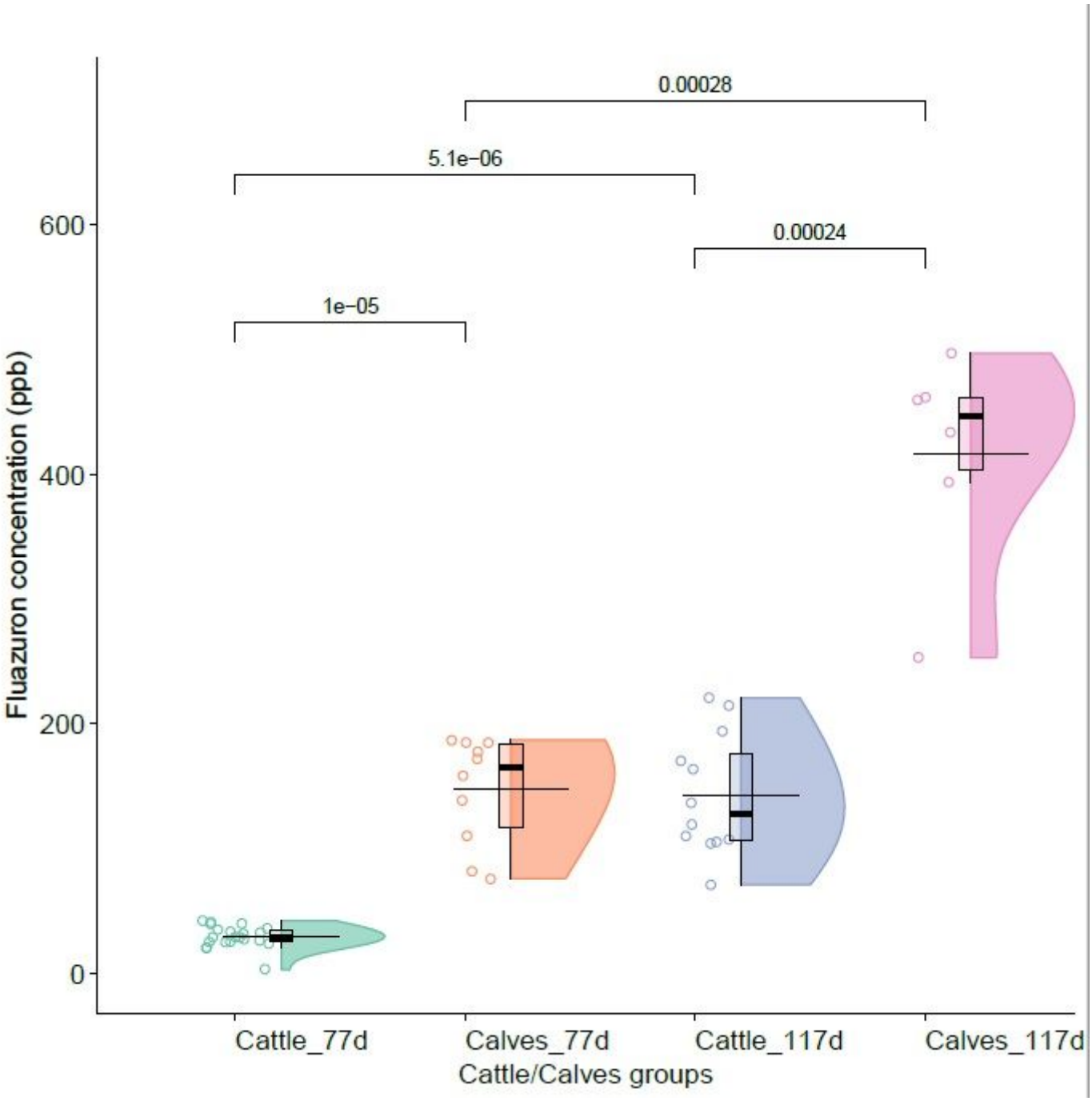

Figure 2

Fluazuron cattle and calves plasma concentration (ppb) before dose repetition in cattle (77 and 117 days from first dose) of a Fluazuron pour-on formulation (2.5 mg/kg, pour-on, 2.5%, Acatak®). Cattle_77d = cattle at 77 day; Calves_77d = calves at 77 day; Cattle_117d = cattle at 117 day; Calves_117d = calves at 117 day. P Value Pairwise (T-test) indicates the significant difference between groups.