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A PRELIMINARY STUDY OF THE BIOLOGICAL AND TOXICOLOGICAL EFFECTS OF TULATHROMYCIN IN SHEEP

STUDIU PRELIMINAR PRIVIND EFECTELE BIOLOGICE ȘI TOXICOLOGICE ALE TULATROMICINEI LA OVINE

C. MOLDOVAN (POP)[1)], A. DREANCĂ[1)], M. MADRU[2)], C. MUREȘAN[1)], I. MARCUS[1)]

ABSTRACT | REZUMAT

Tulathromycin is an antibiotic from the macrolide class used to treat respiratory infections in cattle and swine. Tulathromycin has been studied in the case of an experimental infection with respiratory bacterial pathogens in bovines and calves. The purpose of this study is to evaluate the toxicity of tulathromycin in the case of a young ovine with an approximate age of 1.5 years. The study was performed on 12 clinically healthy Țurcana breed sheep,- divided into 4 equal groups. Tulathromycin was administered subcutaneously in 3 different doses (2.5, 7.5, and 12.5 mg/kg) and intramuscularly in 2.5 mg/kg. Clinical (injection site and body mass, temperature) and paraclinical parameters (CBC and specific biochemistry) were evaluated. Administration of tulathromycin to sheep subcutaneously leads to the appearance of an oedematous, erythematous area, which resolves in 1-2 days. The blood count did not reveal significant statistical changes. Biochemical parameters were influenced differently depending on the dose and site of tulathromycin administration. At first sight, it looks like tulathromycin has a hepatotoxic effect, but in sheep, it must be taken into consideration the deworming treatments and the pathogenesis of fas-iolosis. The administration of tulathromycin didn't cause systemic inflammation. The final objective of this clinical toxicity trial is to take into consideration a possible tulathromycin therapy for some of the respiratory and mammary infections in ovine.

Keywords: tulathromycin, ovine, toxicity study

Tulatromicina este un antibiotic din clasa macrolidelor folosit in tratamentul infecțiilor respiratorii la bovine si porcine. Tulatromicina a fost studiată în cazul infecției experimentale cu diferite bacterii patogene pe sfera pulmonară la bovine adulte și viței. Scopul acestui studiu este de a evalua toxicitatea tulatromicinei în cazul tineretului ovin, cu vârsta aproximativă de 1,5 ani. Studiul a fost efectuat pe 12 oi din rasa Țurcana, clinic sănătoase, împărțite în 4 loturi egale. Tulatromicina a fost administrată subcutanat în 3 doze diferite (2.5, 7.5 și 12.5 mg/kg) și intramuscular 2.5 mg/kg. Au fost evaluați parametrii clinici (locul de injectare și masa corporală, temperatura) și paraclinici (hematologici și biochimici). Administrarea subcutanată a tulatromicinei la oi duce la apariția unei zone edematoase, eritematoase, care se rezolvă în 1-2 zile. Hemoleucograma nu a evidențiat modificări statistice semnificative. Parametrii biochimici au fost influențați diferit în funcție de doza și locul de administrare a tulatromicinei. La prima vedere se observă că tulatromicina are efect hepatotoxic, dar trebuie luate in considerare tratamentele de deparazitare si patogeneza fasciolozei. De asemenea, administrarea de tulatromicină nu a provocat inflamație sistemică. Obiectivul final al acestui studiu clinic de toxicitate este luarea în considerare a unei posibile terapii cu tulatromicină pentru unele dintre infecțiile respiratorii și mamare la ovine.

Cuvinte cheie: tulatromicina, ovine, studiu toxicologic

Tulathromycin is an antibiotic from the macrolide class used in the treatment of respiratory infections in cattle and swine caused by different infectious agents (6). Macrolides are antibacterial agents used on a large scale for treating respiratory ailments and the infection of soft tissues and skin in animals (20). The macrolide class consists in general of bacteriostatic drugs, blocking the synthesis of proteins by the reversible bond to the 50s ribosomal RNA of the susceptible microorganisms. Tulathromycin is a new member of the triamide subclass, those chemical structure is displayed in Fig. 1. It can be retained at the pulmonary level (the main target organ) up to 9 days after administering the single unique doze. Macrolides are in general well tolerated by most species, but in some circumstances, they can cause significant side effects. The most frequent side effects are seen at the gastrointestinal level in the form of nausea, vomiting, abdominal pain, and diarrhoea. Hepatotoxicity, ototoxicity, and cardiotoxicity are less frequent but very serious side effects (2). Recent studies have shown the possi-

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj Napoca, Romania
2) S.C. Profesional Vet S.R.L, Apahida, Cluj, Romania
*) *Corresponding author: alexandra.dreanca@usamvcluj.ro*

bility of tulathromycin having a cardiotoxic effect by raising the concentrations of reactive oxygen species at the cellular level and also affecting ionic potassium, calcium, and clotting factors (8, 9).

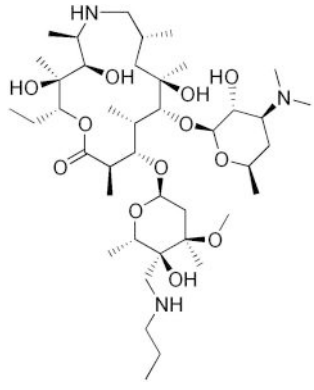

Fig. 1. Chemical structure of tulathromycin (2)

Recently, used in the treatment of pasteurellosis and mycoplasmosis in swine and calves (10, 11), this new generation of macrolides has been studied in terms of therapeutic effectiveness in the case of experimental infection with *Mycoplasma mycoides* subsp. *mycoides* resulting in contagious pleuropneumonia in bovines (16). As regards the type and expansion of the lesions caused, tulathromycin therapy offered protection for 90% of the animals treated in Kenya and, respectively, 98% of the animals treated in Zambia. The same author mentions the capacity of tulathromycin to prevent the spread of infection with *Mycoplasma mycoides*. In another recent study, tulathromycin's capacity to prevent abortions in sheep was determined (19). It mentions the capacity of this macrolide against abortions caused by *Campylobacter jejuni*, so that only 22.22% of the sheep treated with tulathromycin aborted in comparison to the untreated subjects, which aborted in proportion to 77.77%. In contrast, using macrolides in high doses can produce oxidative stress, increase the permeability of the mitochondrial membrane, and make arrhythmogenic events take place at a faster pace (7, 18).

The European Agency for Drugs and Veterinarian Inspections advises administering a single, unique dose of 2.5 mg/kg subcutaneously in bovines and the same dose intramuscularly in swine. In sheep, it is recommended in the treatment of the early stages of infectious pododermatitis (lameness) associated with *Dichelobacter nodosus*, which requires systemic treatment. The injection is recommended in only one dosage: 2.5 mg/kg, intramuscular (https://www.ema.europa.eu/en/documents/product-information/draxxin-epar-product-information_en.pdf).

The same entity mentions that, up to this moment, only a small number of studies regarding the safety of the target animals in regards to tulathromycin are available. Cardiotoxicity with multifocal degenerative affection of the myocardium was observed in 6-month-old calves after the administration of a one dose of 12.5 mg/kg, respectively 15 mg/kg, sq. A considerable rise in the concentrations of creatine phosphokinase and lactate dehydrogenase in all tested doses, starting with the smallest value of 10 mg/kg, was reported. There weren't signs of myocardial toxicity in other studies after the administration of 7.5 mg/kg to cattle aged 4 to 6 weeks and 12.5 mg/kg to 8-months-old cattle. To our knowledge, there are no toxicity or safety clinical studies on ovine. Therefore, the purpose of this study is to evaluate the toxicity of tulathromycin in the case of young ovine with an approximate age of 1.5 years. The focus is to acknowledge the biological effects determined by tulathromycin administration in various ways, both as an immunomodulatory and as an antimicrobial treatment. The final objective of this clinical trial was to implement tulathromycin therapy for some of the respiratory infections in ovine by administering different doses, both subcutaneous and intramuscular. The subcutaneous route is not specific to ovine but is highly used in the field by vets as an alternative to the intramuscular administration of injections of several drugs, such as vaccines; therefore, this route was tested as well. Also, there is no explanation about why the product is recommended for intramuscular use in sheep.

MATERIALS AND METHODS

The study was performed on 12 clinically healthy Țurcana breed sheep, 1.5 years old, in the town of Apahida, Romania. The ovine originated from a homogenous lot and did not come into contact with other animals, having free access to pasture. The subjects were identified based on the number of crotalium. They will be referred based on the last 3 digits of their code for discussing individual parameters. All the procedures carried out in the present project were approved by the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Bioethics Committee (399/21.07.2023).

The ovine was divided into four groups, as follows: Group 1 (n=3): administration of 2.5 mg/kg, sq tulathromycin, in the neck region; Group 2 (n=3): administration of 7.5 mg/kg, sq. tulathromycin, in the tail region; Group 3 (n=3): administration of 12.5 mg/kg, sq., tulathromycin, in the neck region; and Group 4 (n =3): administration of 2.5 mg/kg, i.m. tulathromycin, in the neck region. The site of injection was marked (Fig. 1) for better visualisation of a possible erythema. The animals were weighed, and their body temperature was monitored. Blood samples were taken for haematological and biochemical investigations. The blood sampling took place on day 0, before the admi-

nistration, on day 1, 24 hours after the administration, on day 14, and on day 21 post-administration.

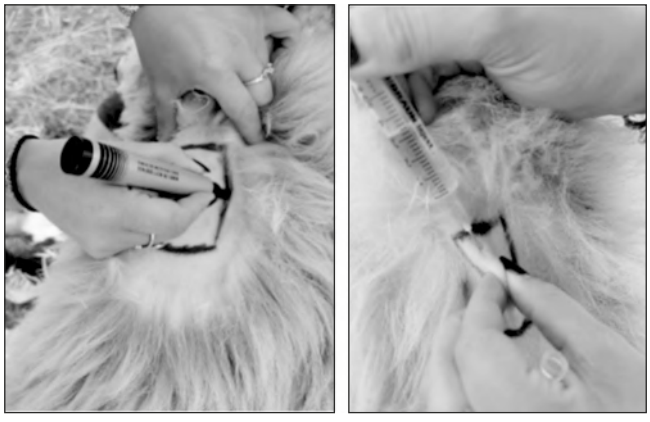

Fig. 2. Marking the site of injection and injecting subcutaneously with tulathromycin

The blood count was performed using the automated analyser Abacus Junior Vet 5 Diff, observing the following: leucocyte parameters (WBC, lymphocytes, medium cells, and granulocytes), erythrocyte parameters (RBC, HCT, and HB), and platelet parameters (PLT). The biochemical investigation was performed using the spectrophotometric analyser Touch UV-VIS Screen (Diagnostics Hospitex, Frienze, Italy). The results were set as the average ± standard deviation; they were obtained by performing one-way ANOVAs and multiple regression tests. For all the applied tests, it was taken into consideration the level of significance $p<0.05$ (95% confidence interval), respectively: $p < 0.05$ but > 0.01 –significant difference; $p < 0.01$ but > 0.001 – very significant; $p < 0.001$ highly significant; $p > 0.05$ insignificant difference.

RESULTS AND DISCUSSIONS

After the subcutaneous administration both in the neck and tail regions, on the second day, oedema appeared in the region (Fig. 3). Any other side effects or reactions were not observed. In contrast to our study, in sheep, transient signs of discomfort (head shaking, injection site rubbing, and backward walking) are reported to be very common after intramuscular injection. These signs resolve within a few minutes.

As previously stated, the temperature and weight of the animals were monitored and varied according to Table 1 and Fig. 5. Both parameters were recorded on days 1, 7, 14, and 21 post-injections. No significant statistical changes were observed within the studied groups concerning the body temperature or the body weight-monitoring period.

Fig. 3. The appearance of post-injection oedema

The weight variation of the individuals included in the study during the five time periods of monitoring is presented in Fig. 4. The average weights of the individuals in group 1 had a gradual decrease after the administration of tulathromycin, so that from an average weight of 44.6 kg on day 1, an average of 38.16 kg was reached on day 21 post-administration, thus recording a decrease in body mass by 14.42%. Looking at group 2, the weight of the individuals did not vary significantly; on day 1, the average recorded was 41.866 kg, and on day 21, we registered 43.266 kg, with an increase of 3.35%. Group 3 averaged 43.01 kg on day 1, reaching 41.96 kg on day 21, thus registering a weight loss of 2.44%. It was the last group of individuals that maintained their weight, with only a 0.95% difference between days 1-21 (38.56 kg vs. 38.93 kg). The changes were not statistically significant.

In Table 2, the variation of the average haematological parameters recorded is shown. In all animals, after 24 hours post-administration of tulathromycin,

Table 1

Body temperature* variation at 1-, 7-, 14-, and 21 days' post-injection of tulathromycin

	DAY 1	DAY 7	DAY 14	DAY 21
Group 1	39.866±0.05	39.8±0.26	39.6±0.34	39.733±0.25
Group 2	39.933±0.11	39.666±0.28	39.6±0.26	40.066±0.20
Group 3	39.966±0.64	39.9±0.20	39.8±0.10	39.833±0.05
Group 4	39.833±0.15	39.766±0.05	39.5±0.30	39.8±0.26

* The normal physiological temperature in sheep is 38.5 °C - 40.0°C (18)

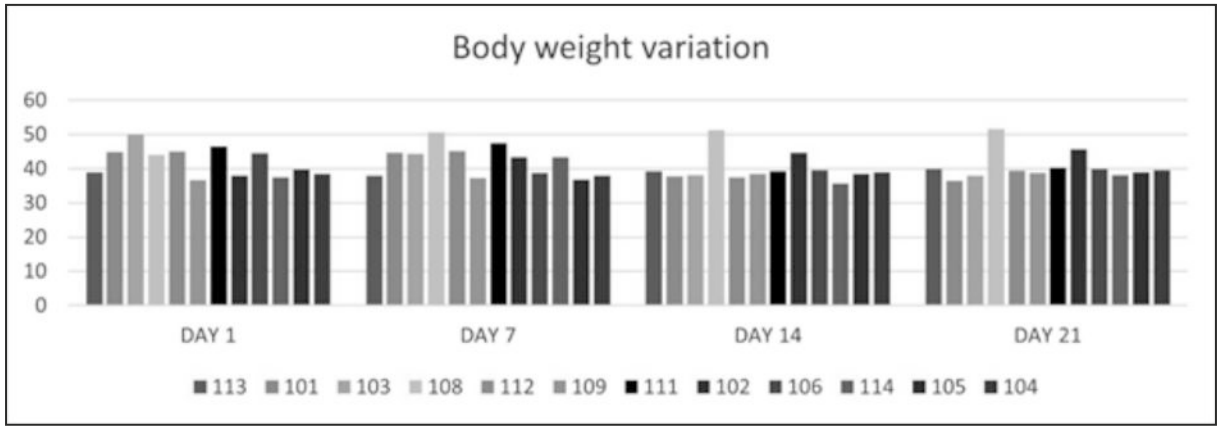

Fig. 4. Body weight variation in dynamics over the study period

leucocytosis with lymphocytosis (13.44± 5.36) was observed. In the remaining periods, the leukogram values were found to be in the normal range of the species (4-12 × 10^9/L). Interestingly, an acute, nonspecific immune modulation can be suspected.

Throughout the study, the physiological limits were not exceeded regarding the values recorded in monocytes and eosinophils. The erythrocyte values did not register major variations (day 1: 9.99 × 10^{12}/L; day 21: 10.23×10^{12}/L), contrary to the data published by other researchers who mentioned tulathromycin as having an effect of decreasing the value of red blood cells, haematocrit, and haemoglobin (3). Platelets, increased post-administration until day 7, following a downward trend until day 21 and falling within the physiological limits of the species.

The biochemistry revealed changes in the value of different enzymes, and we grouped their evolution in Figures 5, 6, and 7 along with Table 2, depending on the organ specificity. The hepatic enzyme, GLDH, presents a rise already from day 0, within the physiological limits (≤60 IU/l), before tulathromycin administration, which may suggest pre-existing liver disease, possibly caused by parasitic infestation or recent deworming of individuals. (3, 14, 15) Similar data were obtained in a study where nutritional deficiency of copper, cobalt, and selenium was demonstrated to affect sheep. (12). After administration of tulathromycin, a statistically significant increase in GLDH is observed only in group 3 (40.33 IU/L), which received a dose of

Table 2

Results of the blood count in time-dynamic*

	WBC	LYM	MON	NEU	RBC
Day 0	11.7267± 1.2410	8.8822± 0.7351	0.0578± 5.68	2.02± 0.2516	10.07± 0.1663
Day 1	13.484± 1.6390	10.807± 1.2968	0.07± 0.00717	2.795± 0.3260	10.007± 0.2543
Day 7	11.10± 1.551	8.81± 1.479	0.05± 0.0055	2.53± 0.3074	10.79± 0.1397
Day 14	11.46± 2.31	8.744± 1.341	0.058± 0.021	2.674± 0.438	10.062± 0.421
Day 21	8.19 ± 1.03	6.45± 0.46	0.045± 0.007	1.72± 0.22	10.13± 0.34
	HGB	**HCT**	**MCV**	**MCH**	**PLT**
Day 0	11.3111± 0.3112	30.3644± 0.7032	29.4444± 0.5235	11.14± 0.2273	119.67± 20.44
Day 1	10.65± 0.3312	30.398± 0.9014	29.7± 0.584	10.76± 0.1251	120.2± 24.25
Day 7	11.17± 0.2880	32.06± 0.7852	29.8± 0.412	10.56± 0.2118	236.5± 17.31
Day 14	11.6± 0.489	31.624± 1.102	29.0± 1.732	11.24± 0.228	196.8± 9.178
Day 21	11.63± 0.23	30.41± 0.83	29.67± 0.61	11.63± 0.41	103.67± 26.59

*The physiological values in sheep include a range in between WBC: 4 – 8 × 10^9/L, LYM: 2 – 9 × 10^9/L, MON: 0 – 0.75 × 10^9/L, NEU: 0.07 – 0.6 ×10^9/L, RBC: 9 – 15 × 10^{12}/L, HGB: 9 – 15 g/L, HCT: 27 – 45%, MCV: 28 – 40 g/dL, MCH: 8 – 12 g/dL, MCHC: 31 – 35 g/dL, PLT: 100 – 800 × 10^9/L (21)

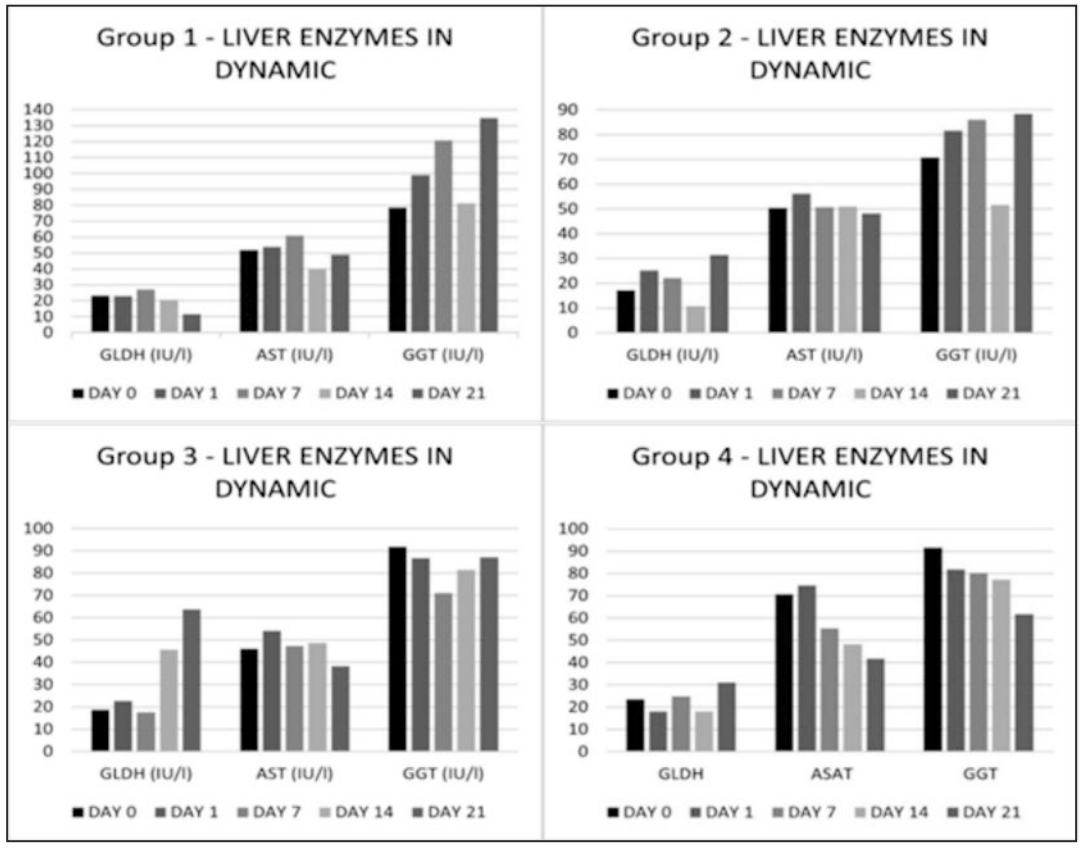

Fig. 5. Liver enzymes in dynamic

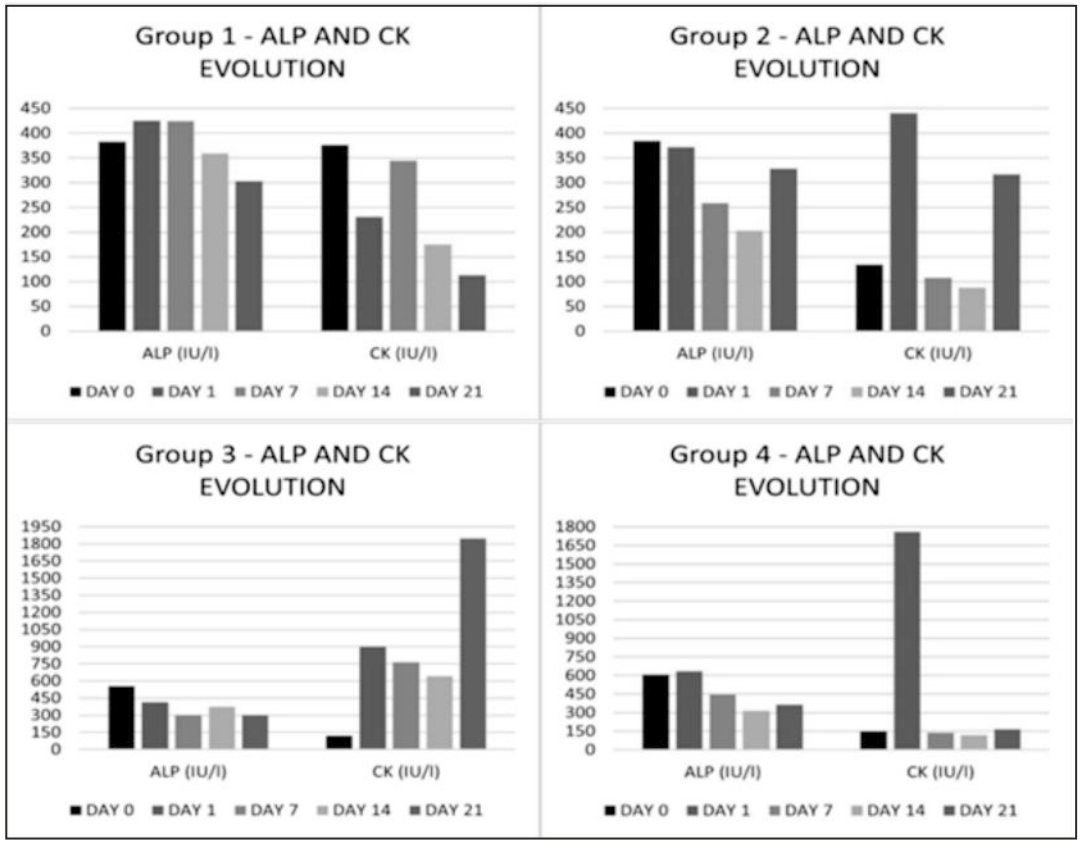

Fig. 6. The evolution of ALP and CK enzymes

12.5 mg/kg, subcutaneous, suggesting the hepatotoxicity of this antibiotic in a high dosage, 5 times higher than the recommended dose. ASAT was kept within physiological limits throughout the experimental period, while GGT, being an enzyme localised in the liver at the level of the bile ducts, which is found in cholestasis syndromes, was elevated from the beginning throughout the study period in all the groups studied (4). An improvement in the value of the ALP enzyme is observed concerning the administration of tulathromycin. In the beginning, the value is much higher than the maximum physiological limit allowed for this species; it decreases (323.69 IU/L) dynamically and approaches the allowed range (70.0 – 390 IU/L) (17). As stated before, in large animals, GGT and ALP have been reported to be increased in times of cholangiohepatitis or cholestasis (3, 13). Since in our experimental groups, except group 3, GLDH and AST are in the normal ranges, the results are consistent with an existing biliary disease in these groups. Hepatotoxicity of tulathromycin was excluded for groups 1, 2, and 4.

In group 3, an increase in the CK enzyme is observed throughout the study, including 21 days after administration. Interestingly, in group 4, the administration of tulathromycin by the intramuscular route directly affects the CK enzyme because it increases statistically significantly on day 1 at 24 h post-administration (1759.53 ± 450 IU/L). Later, it is observed that this increase is transitory because on days 7, 14, and 21, it normalises and subsides within physiological limits (15). According to previous studies carried out in sheep (5), rabbits (9), or mice (1), the administration of tulathromycin has a certain level of cardiotoxicity, affecting the level of specific parameters and the histological appearance of cardiac tissue (1). We can mention that at least the administration of a high dose (12.5 mg/kg, s.c.) and the intramuscular administration of tulathromycin at the recommended dose (2.5 mg/kg, i.m.) affect the cardiac or muscular system.

Urea and creatinine do not present statistically significant values. The values remain within the normal limits (15), as can be observed below in Fig. 7. The serum calcium value registered decreases 21 days post-tulathromycin administration. Plasma calcium concentrations are influenced by food supply; this decrease does not correlate with any other modified parameter to indicate any pathology.

Total protein exceeds the physiological range (6 – 7.9 g/dL) only in group 4 at 7 days post-administration based on the value of the globulins, which also surpasses the normal physiological range (17). We assume that specific IgG has increased, but the same correlation can be seen at 14 days in group 1. An increase in total proteins, globulins, and a mild increase in albu-

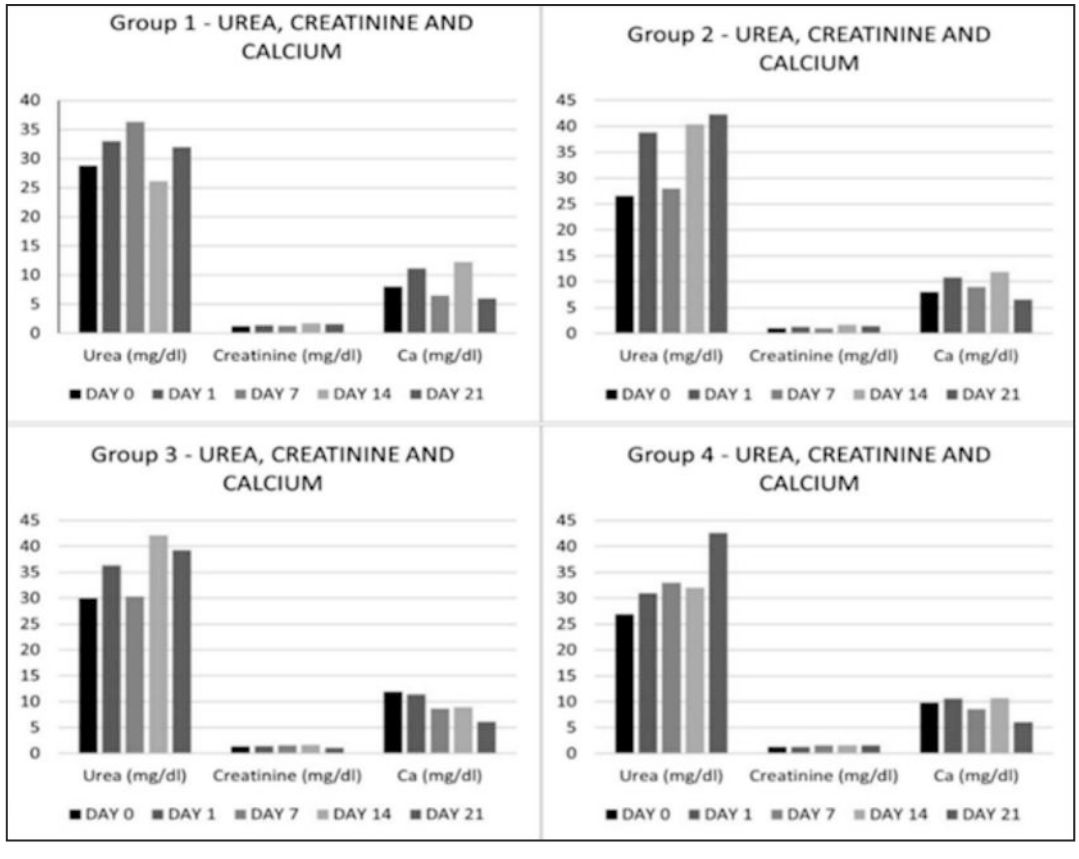

Fig. 7. Urea, creatine, and calcium

Table 3

The effect of tulathromycin administration on the biochemical variations in the blood proteins of Turcana sheep

Group	Total protein	Albumin	γ-Globulin	Haptoglobin
DAY 0				
1 (n = 3)	6.276 ± 0.642	3.28 ± 0.265	1.62 ± 0.807	0.0106 ± 0.003
2 (n = 3)	5.883 ± 0.656	2.903 ± 0.172	1.243 ± 0.919	0.006 ± 0.004
3 (n = 3)	6.17 ± 1.298	2.74 ± 1.161	1.53 ± 0.219	0.0075 ± 0.003
4 (n = 3)	6.026 ± 0.253	3.143 ± 0.797	1.383 ± 0.090	0.013 ± 0.001
DAY 1				
1 (n = 3)	6.2 ± 0.458	3 ± 0.171	1.363 ± 0.281	0.0105 ± 0.002
2 (n = 3)	6.3 ± 0.265	3.166 ± 0.503	1.356 ± 0.076	0.005 ± 0.005
3 (n = 3)	6.633 ± 0.451	2.716 ± 0.161	1.553 ± 0.348	0.009 ± 0.003
4 (n = 3)	6.233 ± 0.208	2.58 ± 0.452	1.503 ± 0.255	0.012 ± 0.002
DAY 7				
1 (n = 3)	6.003 ± 0.523	2.57 ± 0.184	0.993 ± 0.183	0.0138 ± 0.005
2 (n = 3)	5.766 ± 0.571	2.806 ± 0.198	0.746 ± 0.382	0.0104 ± 0.0002
3 (n = 3)	6.413 ± 0.168	3.136 ± 0.121	1.42 ± 0.100	0.011 ± 0.0004
4 (n = 3)	6.866 ± 0.153	2.453 ± 0.136	1.86 ± 0.049	0.011 ± 0.001
DAY 14				
1 (n = 3)	6.9 ± 0.100	2.96 ± 0.140	1.816 ± 0.275	0.01 ± 0.001
2 (n = 3)	5.8 ± 0.200	2.733 ± 0.076	1.473 ± 0.205	0.011 ± 0.002
3 (n = 3)	6.1 ± 0.153	2.7 ± 0.201	1.2 ± 0.153	0.015 ± 0.006
4 (n = 3)	4.9 ± 0.361	2.44 ± 0.252	1.267 ± 0.049	0.012 ± 0.001
DAY 21				
1 (n = 3)	6.12 ± 0.122	3.07 ± 0.157	1.323 ± 0.116	0.011 ± 0.001
2 (n = 3)	6.02 ± 0.171	2.697 ± 0.299	1.127 ± 0.093	0.015 ± 0.006
3 (n = 3)	5.99 ± 0.276	3.24 ± 0.069	1.243 ± 0.060	0.012 ± 0.001
4 (n = 3)	6.293 ± 0.170	3.09 ± 0.061	1.617 ± 0.031	0.012 ± 0.0003

mins were recorded, as Table 3 states. Haptoglobin is an indicator of infection in sheep; it is thought to be more specific for bacterial infection than parasitic infection (13). Nevertheless, in our study, haptoglobin remains in normal ranges (17), which indicates our subjects are clear of systemic inflammation and bacterial infection, and the administration of tulathromycin does not cause systemic inflammation in any tested doses.

CONCLUSIONS

The administration of tulathromycin at the recommended dose of 2.5 mg/kg subcutaneously does not present toxic effects, neither at a systemic nor at any organ level. The dose of 12.5 mg/kg may result in hepatotoxic and cardiotoxic effects, so we do not recommend this dose to be administered in sheep. The administration of large doses of tulathromycin leads to an increase in the value of creatine kinase in the blood, thus causing cardiac damage. The administration of tulathromycin did not cause systemic inflammation, considering the fact that haptoglobin remained in the physiological range throughout the whole period of the study. The next steps to complete the study and our current limitations are as follows: determination of troponin value to confirm possible cardiac damage following tulathromycin administration in sheep and in vitro study of the effects of tulathromycin on bacterial strains isolated from sheep. Another question remains if the specific immunoglobulins (IG E and IG M) are influenced by the administration of tulathromycin, indicating an immunomodulatory effect. This is the first clinical safety study of tulathromycin in Țurcana breed in Romania, addressing several administration pathways due to the large numbers of veterinarians who use both methods in field medicine. Another original aspect is the biochemical concentration characterization in sheep at 1.5 years old. The most important original aspect is the data acquired from the targeted species, ovine, regarding the biological effects of macrolides in field conditions, for future treatment perspectives, standardised therapy, and not only adjusted non-formal immunomodulatory treatments.

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