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Electrocardiographic, echocardiographic and heart biomarker parameters in sheep experimentally poisoned by *Palicourea marcgravii* (Rubiaceae)[1]

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ABSTRACT.- Cunha I.M., Lessa D.A.B., Carvalho V.A.N., Alencar N.X., Teixeira A.L.S., Chenard M.G., Souza G.N. & Helayel M.J.SA. 2022. **Electrocardiographic, echocardiographic and heart biomarker parameters in sheep experimentally poisoned by *Palicourea marcgravii* (Rubiaceae)**. *Pesquisa Veterinária Brasileira 42:e07097, 2022*. Universidade Federal Fluminense, Av. Almirante Ary Parreiras 503, Santa Rosa, Niterói, RJ 24230-340, Brazil. E-mail: isabellemedvet@gmail.com

The present study aimed to identify and describe cardiac alterations in sheep experimentally poisoned with *Palicourea marcgravii* through analysis of serum cardiac biomarkers (serum troponin I and creatine kinase - CK-MB) and electro and echocardiographic assessments to contribute to a better understanding of the poisoning pathophysiology. *P. marcgravii* is the main plant within a group of 22 species that cause sudden death in Brazil; its toxic principle is sodium monofluoroacetate. Eight healthy crossbreed male sheep, aged between five and twelve months, weighing 14 to 27kg, were evaluated. The animals received 1g kg^{-1} of *P. marcgravii* plants orally. The sheep were evaluated before administering the plant (T0) through electro and echocardiography and blood collection to assess cardiac biomarkers (CK-MB and cTnI). Collections and analyses were repeated every four hours until the animal's death. During the study, there was the presence of extravasation of serum troponin I carried out in a qualitative test, with positive values at time T4, and the serum CK-MB biomarker had a peak at T4 and slightly decreased at T8. The electro and echocardiographic examinations showed that the cause of death in these animals was due to acute heart failure characterized by arrhythmias, tachycardia/ventricular fibrillation, drop in cardiac output, left ventricular (LV) systolic dysfunction by the progressive decrease in the LV ejection fraction (EF), decrease in LV fractional shortening (FS), and decrease in aortic flow velocity and aortic flow gradient. This study seems to be the first to evaluate cardiac alterations in sheep poisoned by *P. marcgravii* through cardiac biomarkers and electro and echocardiographic exams.

INDEX TERMS: Electrocardiogram, echocardiogram, cardiology, sheep, intoxication, poisoning plants, *Palicourea marcgravii*.

RESUMO.- [**Parâmetros eletrocardiográficos, ecocardiográficos e de biomarcadores cardíacos em ovinos intoxicados experimentalmente por *Palicourea marcgravii* (Rubiaceae).**] O presente estudo objetivou identificar e descrever as alterações cardíacas de ovinos intoxicados experimentalmente por *Palicourea marcgravii* através das análises de biomarcadores cardíacos séricos (troponina I sérica e a creatinoquinase - MB) e das avaliações eletro e ecocardiográficas contribuindo para o melhor entendimento da fisiopatologia da intoxicação. *Palicourea marcgravii* é a principal planta dentro de um grupo de 22 espécies que causam "morte súbita" no Brasil e seu princípio tóxico é o monofluoracetato de sódio. Foram utilizados oito ovinos saudáveis machos, sem raça definida, com idades entre cinco e doze meses e peso de 14 a 27kg. Os animais receberam 1g/kg de *Palicourea marcgravii* por via oral. Os ovinos foram avaliados momentos antes da administração da planta (T0) através de eletro e ecocardiograma e coleta de sangue para avaliação dos biomarcadores cardíacos (CK-MB

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e cTnI). As análises e coletas foram repetidas a cada quatro horas até o óbito do animal. Durante o estudo observou-se extravasamento de troponina I sérica realizada em teste qualitativo, com valores positivos já em T4, assim como o biomarcador CK-MB sérico teve seu pico de aumento em T4 e em T8 houve uma leve redução. Aos exames eletro e ecocardiográfico foi possível determinar que a causa do óbito nestes animais ocorreu devido à insuficiência cardíaca aguda caracterizada por arritmias, taquicardia/fibrilação ventricular, queda no débito cardíaco, disfunção sistólica do ventrículo esquerdo (VE) pela diminuição progressiva da fração de ejeção (EF) do VE, diminuição na fração de encurtamento (FS) do VE, diminuição da velocidade do fluxo aórtico e do gradiente do fluxo aórtico. Este é o primeiro estudo que avalia as alterações cardiológicas de ovinos intoxicados por *P. marcgravii* através de biomarcadores cardíacos e exames eletro e ecocardiográficos.

TERMOS DE INDEXAÇÃO: Eletrocardiograma, ecocardiograma, cardiologia, ovinos, intoxicação, *Palicourea marcgravii*.

INTRODUCTION

Palicourea marcgravii is one of the most important plants in Brazil (Nascimento et al. 2018), which has sodium monofluoroacetate (MF) as a toxic principle (Oliveira 1963, Krebs et al. 1994, Moraes-Moreau et al. 1995, Nogueira et al. 2010, Peixoto et al. 2012). This agent generates cardiotoxic effects in bovine cattle (Maxie & Robinson 2007, Nogueira et al. 2010), sheep (Schultz et al. 1982, Peixoto et al. 2010), horses, goats, rabbits, and monkeys (Foss 1948, Humphreys 1988, Parton 2001). It acts on the central nervous system in dogs, rats, and hamsters (Chenoweth & Gilman 1946), culminating in death.

Poisoned animals develop clinical and pathological conditions compatible with acute heart failure without the development of morphological changes in the heart due to the rapid evolution of the disease (Helayel et al. 2012, Tokarnia et al. 2012, Rodrigues 2015, Nascimento et al. 2018, Serodio et al. 2019). The diagnosis must be confirmed through epidemiological data, including the presence of the plant and the consumption by the animal, additionally with the clinical signs of acute cardiac insufficiency and, when present, the renal microscopic lesion of tubular epithelial degeneration (Tokarnia & Döbereiner 1986, Tokarnia et al. 2000, Helayel et al. 2012, Tokarnia et al. 2012).

The use of cardiac markers such as troponin I (cTnI) and creatine kinase MB (CK-MB) has become frequent in human medicine (Borges et al. 2019) and veterinary (Assis et al. 2017) for early detection of myocardial lesions as they are specific biomarkers of this type of lesion (Fagliari & Thiesen 2015). However, there are no studies on the dosage of these markers in sheep poisoned by *P. marcgravii*.

The use of echocardiogram (ECO) and electrocardiogram (ECG) as a diagnostic aid for plant poisoning that leads to heart failure is promising, as there are no studies in the literature on the use of these techniques to assess cardiac alterations in sheep poisoned by *P. marcgravii*. Studies on electrocardiographic patterns and techniques in sheep are scarce (Torío et al. 1997), which further enhances the importance of the present study.

This study aimed to detect and characterize the electrocardiographic and echocardiographic findings, as well as the changes in the concentrations of the troponin I and CK-MB cardiac biomarkers in sheep experimentally poisoned with *P. marcgravii*.

MATERIALS AND METHODS

The study was approved by the Ethics Committee on the Use of Animals (CEUA) of the "Universidade Federal Fluminense" (UFF) under the number 7702030518.

Eight crossbreeds male sheep, aged between five and twelve months, weighing 14 to 27kg, and healthy on clinical (Dirksen et al. 1993) and hematological (Fagliari & Thiesen 2015) examinations were evaluated. The animals received 1g kg^{-1} of fresh *Palicourea marcgravii* plants (identified at the "Instituto de Biologia" of the "Universidade Federal Rural do Rio de Janeiro" - UFRRJ under the code RBR37508), which were manually orally administered as described by Tokarnia et al. (2012).

The sheep were evaluated before the plant administration (T0) through electro and echocardiography (Boon 2011, Filippi 2011, Santilli et al. 2020) and blood collection (Jain 1993, Fagliari & Thiesen 2015) for evaluation of cardiac biomarkers (CK-MB and cTnI). Analyses and collections were repeated every four hours until the animal's death.

Blood samples were placed in tubes without anticoagulant and immediately centrifuged at 700xg for 20 minutes to obtain the serum stored at -20°C. CK-MB analysis was performed using a commercial kit (LabTest®), and cTnI was performed using a rapid test (One Step Troponin I Test Device®; Abon Biopharm Co. Ltd., China), following the manufacturer's recommendations.

The electrocardiographic recording (ECG) of each patient was performed under mechanical restraint in the right lateral decubitus position using a TEB® digital electrocardiograph, according to the technique described by Filippi (2011) and Santilli et al. (2020). The electrodes of the frontal plane leads were placed in the creases of the right and left elbows and knees, the precordial electrodes were placed as follows: RV2 at the height of the fifth intercostal space on the right side close to the sternum; V2 at the height of the sixth intercostal space near the sternum on the left side (below the costochondral junction); V4 at the height of the sixth intercostal space just above the costochondral junction on the left side and V10 at the height of the tenth thoracic vertebra, in the dorsal thoracic region; the place where the electrodes were fixed was moistened with a 70% alcoholic solution to improve the contact.

Heart rate, time and amplitude of the P wave, time and amplitude of the QRS complex, time of the PR interval, QT interval, ST segment, and T wave morphology was evaluated using the frontal plane in the DII lead at a speed of 50mm s^{-1}. The precordial leads were analyzed: RV2, R wave amplitude and T wave morphology, V2 R and S wave amplitudes, V4 R and S wave amplitudes, V10 QRS complex, and T wave morphology.

Transthoracic echocardiography was performed using an M-Turbo system (Fujifilm® SonoSite, Washington, USA) equipped with a 4.0-8.0MHz sectorial transducer (Px10; Fujifilm® SonoSite, Washington, USA) with coupling gel (Mercur®, São Paulo, Brazil) between the fourth and sixth intercostal space on both sides of the chest, following the techniques described and recommended by the Echocardiography Committee of Specialty of Cardiology - American College of Veterinary Internal Medicine (Thomas et al. 1993) and American Society of Echocardiography (Boon 2011). In the echocardiographic evaluation, through the right parasternal

Table 1. Individual values and means of cardiac biochemistry in pre-poisoning (T0) and post- poisoning (T4 and T8) times in sheep experimentally poisoned with *Palicourea marcgravii*

Variable	Time	Animal								Mean and SD	References
		332	333	342	703	806	810	820	821		
CK-MB (U/I)	T0	361.7	408.9	302.5	341.1	333.6	363.4	272.4	273.7	332.2[a] ± 47.34	*
	T4	402.7	412.3	338.9	481.6	337.8	468.7	385.5	361.5	398.6[b] ± 54.53	
	T8	460.1	426.8	284.5	-	346.8	415.1	-	-	386.7[ab] ± 70.42	

[a,b] Means followed by different letters in the rows are statistically different from each other by the t test for paired samples ($p \leq 0.05$); * There are no data in the reference literature for this species; SD = standard deviation, CK-MB = creatine kinase MB; (–) death.

projection, cross-section of the left ventricle, height of the papillary muscles, measurements were obtained from the interventricular septum at the end of diastole (IVSd), interventricular septum at the end of systole (IVSs), left ventricular diameter at the end of diastole (LVIDd), left ventricular diameter during systole (LVIDs), left ventricular posterior wall thickness in diastole (LVPWd), left ventricular posterior wall diameter in systole (LVPWs), left ventricular ejection fraction (EF) and fractional shortening (FS), and aortic root (Ao) and left atrium (LA).

Statistical analyses were performed using the SPSS computer system. The parametric (quantitative) analyses were subjected to the T-test at a 5% significance level. The analysis of non-parametric parameters was carried out using the Friedman test to assess the difference in categorical or qualitative variables between times. In cases where $p \leq 0.05$ was observed, the Wilcoxon test was applied in time pairs to identify the difference.

RESULTS

There was 100% mortality at the end of the study. T0 represents the pre-poisoning moment; at time T4, all animals remained alive; at time T8, three (03) animals died, and the other five animals died later.

The mean CK-MB peaked at T4, with a significant difference between T0 and T4 ($p<0.05$). There was a slight, non-significant decrease in mean CK-MB values at T8; individually, a progressive increase in CK-MB was observed over time in 75% of the sheep (1, 2, 4, 5, 7, and 8), and a moderate decrease between T4 and T8 in 25% of the sheep (3 and 6) (Table 1).

The cTnI analysis showed that 100% of the animals had negative results at T0, 62.5% had positive results at T4, and 100% had positive results at T8, with significant differences between T0 and T4, and T0 and T8 (Table 2).

In the electrocardiographic examination, 37.5% of the sheep (6, 7 and 8) kept the same cardiac axes at all times evaluated, the sheep 6 (12.5%) kept their axis between +60° and +90°, the 7 (12.5%) between -90° and -120°, and the 8 (12.5%) between -60° and -90°; 50% of the animals (1, 3, 4, and 5) had axis variation at all evaluated times; the Animal 2 (12.5%) had axis variation only between T0 and T4 (Table 3).

The increase in heart rate (HR) showed progressive tachycardia over times T4 and T8, and a significant difference between all times (T0, T4, T8) ($p<0.05$) (Table 4).

The means of the P wave segment (s) and P wave amplitude (mV) did not present a significant difference ($p>0.05$) between times. The mean values of the P-R interval (s) decreased from T0 to T4 and then increased at T8, reaching values close to that of T0, with a significant difference between them ($p<0.05$). Contrastingly, the mean wavelength of the QRS complex (s)

Table 2. Non-parametric statistical analysis of troponin I results in pre- poisoning (T0) and post- poisoning (T4 and T8) times of sheep experimentally poisoned with *Palicourea marcgravii*

Troponin I	Time					
	T0		T4		T8	
	N	%	N	%	N	%
Positive	0	0.0a	5	62.5b	5	100b
Negative	8	100	3	37.5	0	0

[a,b] Means followed by different letters in the rows are statistically different from each other by the t-test for paired samples ($p \leq 0.05$).

Table 3. Evaluation of the cardiac axis in the electrocardiogram exam in the pre- poisoning (T0) and post- poisoning (T4 and T8) times of sheep experimentally poisoned with *Palicourea marcgravii*

Animal	Time	Cardiac axis
332	T0	+180°
	T4	0°
	T8	Between +60° and +90°
333	T0	-90°
	T4	Between +60° and +90°
	T8	Between +60° and +90°
342	T0	-120°
	T4	Between +150° and +180°
	T8	-120°
703	T0	- 120°
	T4	+ 60°
806	T0	0°
	T4	-180°
810	T0	Between +60° and +90°
	T4	Between +60° and +90°
	T8	Between +60° and +90°
820	T0	Between -90° and -120°
	T4	Between -90° and -120°
	T8	Between -90° and -120°
821	T0	Between -60° and -90°
	T4	Between -60° and -90°

remained the same at T0 and T4, with a slight increase at T8, with no significant difference (p>0.05). The mean QRS complex amplitude (mV) increased in all animals from T4 onwards, with a significant difference between them (p<0.05) (Table 4).

The mean Q-T interval (s) decreased from T0 to T4, with a significant difference (p<0.05) and increased slightly from T4 to T8, with no significant difference (p>0.05); the increase in HR generates a decrease in the Q-T interval (s), which are inversely proportional (Table 4). The "torsades de pointes" phenomenon was observed at T8, with the occurrence of tachycardia/ventricular fibrillation. Individually, there was a progressive decrease in the Q-T interval, as shown in Table 5.

The precordial leads RV2 (RmV), V2 (RmV), and V4 (RmV) had the mean R wave amplitude increased at T8 but without significant difference (p>0.05) (Table 4).

The means of precordial leads V2 (SmV) and V4 (SmV) that measure the amplitude of the S wave decreased over time, with a significant difference when comparing T0 to T8 (p<0.05) for V2 (SmV) and no significant difference (p>0.05) for V4 (SmV) (Table 4). Individually, these values show relevant changes, as described in Table 6.

Data referring to the echocardiographic assessment are shown in Table 7. The mean HR was 124.4 bpm at T0, remaining within the reference parameters for the species. It increased 43.4% at T4, with a mean of 178.4 bpm, considered tachycardia. At T8, HR increased 77.3% compared to T0 and 23.6% compared to T4, with a mean of 220.6 bpm and a significant difference between times. Although there was no statistical difference, the isovolumetric relaxation time (IVRT) decreased by 8.75ms at T4 and 20.5ms at T8. Pulmonary flow velocity (PFV) decreased over time, with a significant difference between T0 and T4, and between T4 and T8, with T8 showing no difference from T0 (p<0.05). The pulmonary flow gradient (PFG) also decreased over time, but with a significant difference only at T4 (p<0.05). The mitral E to mitral A wave ratio (E/A) was not possible to measure at T8 because the animals were very tachycardic, generating wave fusion at the time of the echocardiographic examination. As the mitral E wave velocity increases, the mitral A wave velocity

Table 4. Means and standard deviations of the parameters analyzed in the electrocardiography exam in the pre- poisoning (T0) and post- poisoning (T4 and T8) times of sheep experimentally poisoned with *Palicourea marcgravii*

ECG	Time		
	T0 (n=8)	T4 (n=8)	T8 (n=5)
	Mean and SD	Mean and SD	Mean and SD
HR (bpm)	110.37a ± 19.61	179.75b ± 50.07	185.20c ± 34.68
P(s)	0.022a ± 0.007	0.025a ± 0.009	0.02 ± 0
P(mV)	0.12a ± 0.04	0.13a ± 0.05	0.12 ± 0.04
P-R(s)	0.107b ± 0.014	0.090a ± 0.018	0.108ab ± 0.01
QRS(s)	0.037a ± 0.007	0.037a ± 0.007	0.040a ± 0
QRS(mV)	0.125a ± 0.046	0.200b ± 0.075	0.200ab ± 0.1
Q-T(s)	0.275b ± 0.027	0.180a ± 0.078	0.188a ± 0.054
RV2(RmV)	0.125a ± 0.046	0.125a ± 0.046	0.160a ± 0.054
V2(RmV)	0.100a ± 0	0.100a ± 0	0.160a ± 0.894
V2(SmV)	0.400b ± 0.232	0.275ab ± 0.158	0.220a ± 0.164
V4(RmV)	0.112a ± 0.035	0.150a ± 0.075	0.160a ± 0.054
V4(SmV)	0.175a ± 0.088	0.125a ± 0.046	0.120a ± 0.04

[a,b,c] Means followed by different letters in the rows are statistically different from each other by the t-test for paired samples (p≤0.05); n = Sample number/ live sheep, SD = standard deviation, HR = heart rate, P(s) = P wavelength, P(mV) = P wave amplitude, P-R(s) = P-R interval, QRS(s) = QRS wavelength, QRS(mV) = QRS wave amplitude, Q-T(s) = Q-T interval, RV2(RmV) = precordial lead-R wave amplitude, V2(RmV) = precordial lead-R wave amplitude, V2(SmV) = precordial lead-S wave amplitude, V4(RmV) = precordial lead-R wave amplitude, V4(SmV) = precordial lead-S wave amplitude.

Table 5. Individual values of the Q-T interval (s) in the pre- poisoning (T0) and post- poisoning (T4 and T8) times of sheep experimentally poisoned with *Palicourea marcgravii*

Animal	Q-T(s)		
	T0 (n=8)	T4 (n=8)	T8 (n=5)
332	0.28	0.26	0.22
333	0.28	0.28	0.24
342	0.28	0.28	0.18
703	0.28	0.14	-
806	0.26	0.1	-
810	0.32	0.14	0.2
820	0.28	0.12	0.1
821	0.22	0.12	-

Q-T(s) = Q-T Interval; (–) death.

Table 6. Individual values of precordial leads V2 (SmV) and V4 (SmV) in pre- poisoning (T0) and post- poisoning (T4 and T8) times of sheep experimentally poisoned with *Palicourea marcgravii*

Animal	V2(SmV)			V4(SmV)		
	T0	T4	T8	T0	T4	T8
332	0.3	0.3	0.2	0.1	0.1	0.1
333	0.3	0.1	0.1	0.3	0.1	0.1
342	0.9	0.3	0.5	0.1	0.2	0.1
703	0.6	0.6	-	0.2	0.1	-
806	0.2	0.3	-	0.2	0.2	-
810	0.3	0.2	0.1	0.1	0.1	0.1
820	0.3	0.3	0.2	0.3	0.1	0.2
821	0.3	0.1	-	0.1	0.1	-

V2(SmV) = Precordial lead-S wave amplitude, V4(SmV) = precordial lead-S wave amplitude; (–) death.

decelerates, denoting an inversely proportional correlation. Thus, it was impossible to determine the A wave at T8 and the E to A wave ratio (Table 7).

The results of the Doppler echocardiographic assessment performed at T4 and T8 indicated acute heart failure caused by poisoning by *P. marcgravii*, as the sheep had tachycardia, left ventricular (LV) systolic dysfunction characterized by a progressive decrease in LV ejection fraction (EF) (T0 = 66.50%, T4 = 55.13%, T8 = 50.8%), decrease in LV fractional shortening (FS) (T0 = 35.54%, T4 = 29.09%, T8 = 25.96%), and decrease in aortic flow velocity (T0 = 91.03%, T4 = 70.34%, T8 = 65.94%) and aortic flow gradient (T0 = 3.45%, T4 = 2.05%, T8 = 1.81%). The impairment in cardiac function generated cardiac remodeling; eccentric hypertrophy was observed from the T4 phase onwards, which is explained by the increase in left atrium (T0 = 2.12cm, T4 = 2.51cm, T8 = 2.86cm) and left atrium to aorta ratio (T0 = 1.20, T4 = 1.51, T8 = 1.84), and the increased distance of the mitral valve leaflet in relation to the interventricular septum (SSPE) (T0 = 0.42, T4 = 0.51, T8 = 0.68). The SSPE gradually increased over time, but a significant difference was only observed after T8 (p<0.05). Although the SSPE showed this dilation, the LV diameter at the diastole did not present a significant difference (p>0.05) (Table 7).

DISCUSSION

This study seems to be the first to demonstrate cardiac alterations identified through electro and echocardiographic examinations and with measurements of cardiac biomarkers - creatine kinase-MB (CK-MB) and troponin I (cTnI) - in sheep poisoned by *Palicourea marcgravii*, which contributes to a better understanding of the poisoning pathophysiology.

The increase in CK-MB with an early peak in the first post-poisoning assessment (T4) confirms the presence of cardiac damage, as it is a specific enzyme of the cardiac muscle (Aktas et al. 1993, Kramer & Hoffmann 1997). A slight decrease in CK-MB occurred at T8; the cardiac arrest remained despite being a short-acting enzyme with a return to normality within 48 hours (Fagliari & Thiesen 2015). These findings differ from Barbosa (2016), who reported no change in CK-MB concentrations in sheep chronically poisoned by *P. marcgravii*, probably due to a long interval between the animal evaluations (twice a week). Isoenzyme is important for determining the affected site and is commonly used in human medicine. However, it is not yet used in the routine practice of veterinary (Yonezawa et al. 2010, Fagliari & Thiesen 2015), mainly in farm animals.

The serum cTnI detected from T4 onwards confirms the enzyme's efficacy in the early detection of cardiac damage

Table 7. Means and standard deviations of the parameters analyzed in the echocardiography exam at times T0 (pre-poisoning), T4, and T8 (post-poisoning) of sheep experimentally poisoned with *Palicourea marcgravii*

ECHO	Time		
	T0 (n=8)	T4 (n=8)	T8 (n=5)
	Mean and SD	Mean and SD	Mean and SD
HR (bpm)	124.4^{a} ± 24.4	178.4^{bc} ± 635	220.6^{c} ± 42.9
IVSd (cm)	0.69^{a} ± 0.17	0.70^{a} ± 0.19	0.73^{a} ± 0.17
IVSs (cm)	0.93^{a} ± 0.21	0.84^{a} ± 0.2	0.84^{a} ± 0.23
LVIDd (cm)	2.83^{a} ± 0.22	2.65^{a} ± 0.25	2.83^{a} ± 0.09
LVIDs (cm)	1.83^{a} ± 0.2	1.89^{a} ± 0.49	2.10^{b} ± 0.4
LVPWd (cm)	0.76^{a} ± 0.21	0.70^{a} ± 0.2	0.69^{a} ± 0.14
LVPWs (cm)	1.14^{a} ± 0.23	1.00^{a} ± 0.22	1.14^{a} ± 0.27
EF (%)	66.50^{a} ± 7.05	55.13^{a} ± 21.36	50.80^{a} ± 18.24
SF (%)	35.54^{a} ± 5.63	29.09^{a} ± 13.62	25.96^{a} ± 12.87
Ao (cm)	1.77^{a} ± 0.14	1.77^{ab} ± 0.15	1.59^{b} ± 0.21
LA (cm)	2.12^{a} ± 0,29	2.51^{a} ± 0.48	2.86^{a} ± 0.5
LA/Ao	1.20^{a} ± 0.16	1.51^{b} ± 0.32	1.84^{ab} ± 0.46
MEWV (cm s^{-1})	57.28^{a} ± 8.74	76.90^{a} ± 39.78	105.00^{a} ± 37
MAWV (cm s^{-1})	56.95^{a} ± 16.7	56.14^{a} ± 29.2	-
E/A mitral	1.05^{a} ± 0.21	1.40^{a} ± 0.43	-
EWDT (ms)	107.5 ± 42.51	105.63 ± 50.17	90 ± 18.37
PFV (cm s^{-1})	74.91^{a} ± 12.87	51.74^{b} ± 16.05	50.60^{ac} ± 17.23
PFG (mmHg)	2.30^{a} ± 0.77	1.16^{b} ± 0.63	1.12^{a} ± 0.65
AoFV (cm s^{-1})	91.03^{a} ± 19.65	70.34^{b} ± 14.55	65.94^{b} ± 14.79
AoFG (mmHg)	3.45^{a} ± 1.52	2.05^{b} ± 0.78	1.81^{b} ± 0.78
IVRT (ms)	77.50^{a} ± 24.78	68.75^{a} ± 41.98	57.00^{a} ± 9.08
SSPE (cm)	0.42^{a} ± 0.16	0.51^{a} ± 0.12	0.68^{b} ± 0.24

^{a,b,c} Means followed by different letters in the rows are statistically different from each other by the t test for paired samples (p≤0.05); n = Sample number/live sheep, SD = standard deviation, HR = heart rate, IVSd = interventricular septum at the end of diastole, IVSs = interventricular septum at the end of systole, LVIDs = left ventricular diameter during systole, LVIDd = left ventricular diameter during diastole, LVPWd = left ventricular posterior wall thickness in diastole, LVPWs = left ventricular posterior wall thickness in systole, EF = ejection fraction, SF = shortening fraction, Ao = aorta, LA = left atrium, LA/Ao = left atrium to aorta diameter ratio, MEWV = mitral E wave velocity, MAWV = mitral A wave velocity, E/A Mitral = E to A wave ratio, EWDT = E wave deceleration time, PFV = pulmonary flow velocity, PFG = pulmonary flow gradient, AoFV = aortic flow velocity, AoFG = aortic flow gradient, SSPE = mitral leaflet to interventricular septum distance, IVRT = isovolumetric relaxation time.

caused by *P. marcgravii.* It can be easily incorporated into the routine of farm animals, as it is a rapid test that generates immediate results, facilitates field diagnosis, and is low-cost (on average, R$ (BRL) 8.00 per test). No studies on qualitative troponin I dosages in animals poisoned by *P. Marcgravii* were found. Normal cTnI values in ruminants range from 0.005 to 0.09ng mL^{-1} (Fartashvand et al. 2013). The cTnI One Step Troponin I Test Device (Whole Blood/Serum/Plasma)® (kit used) does not detect less than 0.5ng mL^{-1}; thus, the animals positive for the test had values above 0.5ng mL^{-1}. Cardiac troponins have been gaining attention as highly specific markers of cardiac injury (O'Brien 2008). The structure of these troponins is very similar between species (O'Brien et al. 1997), and its release into the circulation is irreversibly related to areas of cardiomyocyte necrosis, and increases in its concentration are proportional to the severity of the injury (Collinson et al. 2001).

There is no standard cardiac axis for sheep species. However, studies have shown considerable variation in the cardiac axis within the species, which is sometimes affected by physiological factors such as carcass conformation, age, sex, rumen filling and decubitus change (Tajik et al. 2015). However, some diseases that lead to an overload of the right ventricle, such as edema and pulmonary hypertension, as seen in the animals in the present study, may be factors affecting this variable (Ahmed & Sanyal 2008); this explains the results obtained in this study. The animals did not have a cardiac axis pattern at T0, and possibly there was not enough time after poisoning to characterize a pattern of cardiac axis alteration, which explains the significant cardiac axis alterations in some animals.

The electrocardiographic examination showed progressive tachycardia, which indicates impaired cardiac function caused by poisoning. It is a similar result to those observed for poisoning by sodium monofluoroacetate (MF) in sheep (Schultz et al. 1982), cattle poisoned by *P. marcgravii* (Rodrigues 2015), and sheep poisoned by *M. rigida* (Lago et al. 2009). The basal electrocardiographic parameters of sheep identified significant variability in the morphology of the QRS complex, and the P wave was present and positive in 100% of the cases. The T wave varied, being positive (71.9%) or biphasic (28.1%). The QT interval had a mean duration of 0.262 seconds, and the mean heart rate was 119 beats per minute (Torío et al. 1997).

Although there was no significant difference in time and amplitude of the P wave, they increased over time. The time of the P wave by the interpretation of the electrocardiographic examination means changes concerning the left atrium (LA) and an increase in the amplitude of the P wave concerning the right atrium (RA), thus determining a sinus tachycardia due to the increase in HR. It occurs due to increased electrical activity, characterizing a right atrial overload. This cardiac systolic dysfunction and tachycardia occurred to maintain compensated physiological activities (Filippi 2011, Santilli et al. 2020). One of the hypotheses is that these animals probably had hypotension due to poisoning and the compensation mechanism was tachycardia.

The P-R interval (s) change occurred because of increased HR over time. This change can occur due to preexcitation syndrome, ectopic atrial rhythm, multifocal atrial tachycardia, or a junctional rhythm (Thaler 2019). In healthy sheep, age and sex affect the results obtained through ECG (Tajik et al. 2015). However, considering that this study was carried out with animals of similar ages and same-sex, changes in HR and consequent changes in P-R interval (s) were generated by the poisoning by *P. marcgravii.*

The increase in the amplitude of the QRS complex combined with the increase in HR occurred to compensate for the systolic dysfunction due to possible hypotension, probably due to the volume overload observed on the echocardiogram (eccentric-type hypertrophy). The QRS complex is related to the volume of the left ventricle (LV) (Filippi 2011, Santilli et al. 2020). This overload of ventricular activity as a function of volume is a physiological response to muscle damage and consequently generates an increase in HR (Filippi 2011, Santilli et al. 2020).

Initially (T0 × T4), the Q-T wave length decreased, indicating electrolyte disturbance, ischemia, or digitalis action (Friedmann et al. 2011). Later (T4 x T8), there was an increase in Q-T waves, an alteration previously reported in cases of MF poisoning, especially in the presence of hypocalcemia (Hayes Jr. & Laws 1991, Chi et al. 1996). In addition, the increase in HR generates a decrease in the Q-T complex, which is inversely proportional (Filippi 2011, Santilli et al. 2020).

Sinus tachycardia was observed, an arrhythmia less evident than that observed by Rodrigues (2015), who found predominant arrhythmia with multifocal extrasystoles and ventricular arrhythmia. Contrastingly, Lago et al. (2009) found no arrhythmias. Studies on the effects of *Pachystigma pygmaeun* (Rubiaceae) in sheep detected changes in the ST segment associated with acute myocardial ischemia, significant changes in cardiac function translated by ventricular extrasystoles, and an increase in T wave amplitude associated with arrhythmias (Van Der Walt et al. 1990). When evaluating the effects of MF on the electrocardiogram of sheep, a reduction in T wave amplitude, PR interval prolongation, and ventricular fibrillation was identified (Schultz et al. 1982). Animals poisoned by MF may have arrhythmias caused by several factors, such as metabolic acidosis, severe electrolyte imbalance, and accumulation of citrate and lactate due to blockage of the Krebs Cycle at the aconitase site (Gal et al. 1956). These factors result in cardiotoxic effects, such as cardiac arrhythmias with ventricular tachycardia, ventricular fibrillation, and cardiac arrest (Collicchio-Zuanaze et al. 2006). Cattle poisoned by *P. marcgravii* showed cardiotoxic effects, with multifocal arrhythmias, especially those of ventricular origin (Rodrigues, 2015).

The ECG revealed severe ventricular arrhythmia at T8, with a tachycardia/ventricular fibrillation called "torsades de pointes", the only work with sheep poisoning by *P. marcagravii* that had the opportunity to monitor this event. "Torsades de pointes" is characterized by fast, irregular QRS complexes, which appear to be twisting around the baseline of the ECG. This arrhythmia may spontaneously cease or degenerate into ventricular fibrillation. It causes significant hemodynamic compromise and death (Filippi 2011, Santilli et al. 2020). In these patients, an infusion of 2g of magnesium sulfate intravenously (IV) can be tried for 1 to 2 minutes, with a second dose applied 5 to 10 minutes later. IV lidocaine may also be effective (Zipes et al. 2006, Neumar et al. 2010, Gonzalez et al. 2013, Martins et al. 2013).

In the echocardiographic evaluation, HR showed a noticeable increase at T4, above normality, characterizing sinus

tachycardia, as observed in sheep and bovine cattle poisoned by *M. rigida* and *P. marcgravii*, respectively (Lago et al. 2009, Rodrigues 2015). As the systolic function was impaired, the cardiac pump function was also impaired, thus raising the HR to compensate for the ejected volume (Boon 2011).

The T4 and T8 results indicated heart failure caused by poisoning by *P. marcgravii*, as the sheep presented tachycardia, left ventricular (LV) systolic dysfunction characterized by a progressive decrease in the LV ejection fraction (EF) (the ability of the left ventricle to contract during each beat to pump the blood properly), decrease in LV fractional shortening (FS) (percentage change in the dimension of the left ventricular cavity that occurs in systole), and decrease in aortic flow velocity (the speed at which blood passes through the aorta artery) and aortic flow gradient (pressure at which blood passes through the aorta artery) (Boon 2011). These alterations were also observed in sheep poisoned by *M. rigida* and cattle poisoned by *P. marcgravii* (Lago et al. 2009, Rodrigues 2015).

The impairment in cardiac function generated cardiac remodeling; eccentric type hypertrophy was observed from the T4 phase onwards; this is justified by the increased left atrium, AE/Ao ratio, and mitral leaflet to interventricular septum distance (SSPE). These parameters have never been evaluated in previous studies. According to Boon (2011), the SSPE is the distance from the mitral valve leaflet at the time of its opening concerning the interventricular septum; thus, there was a gradual increase in this distance over time, indicating eccentric-type hypertrophy.

The mitral E to A wave ratio (E/A) was not possible to measure at T8 because the animals were very tachycardic, which generated wave fusion at the time of the echocardiographic examination. As the mitral E wave velocity increases, it slows the mitral A wave velocity, presenting an inversely proportional correlation; thus, it was impossible to determine the T8 of the A wave and the E to A wave ratio. The E wave passively measures the LV filling by pressure difference. Wave A measures atrial contraction, which corresponds to 30% of ventricular filling, and it is the active filling of the LV by atrial contraction (Boon 2011). The A wave reading was impossible due to the increase in HR, which does not mean it does not exist.

Regarding the isovolumetric relaxation time, there was a reduction between T4 and T8 due to increased HR. This parameter had never been measured in previous studies. The isovolumetric relaxation time is the time between atrial flow and ventricular passive filling, which occurs between a systole and diastole cycle (Boon 2011).

The decrease in pulmonary flow velocity and pressure gradient characterizes a probable RV systolic dysfunction, as it occurred in the LV. There is a right ventricular cardiac output due to a decrease in blood velocity, leaving the pulmonary artery towards the lung to be oxygenated. Left ventricular systolic dysfunction was observed, and the same occurred in the RV. Consequently, the pressure gradient decreased. In studies with sheep and cattle, no abnormalities related to blood flow were found (Lago et al. 2009, Rodrigues 2015).

CONCLUSIONS

Poisoning by *Palicourea marcgravii* causes damage to cardiac muscles, leading to acute heart failure with extravasation and increased serum levels of creatine kinase-MB (CK-MB) and troponin I (cTnI). These enzymes can be used to reveal early cardiac damage in sheep.

The electro and echocardiographic exams efficiently determine the beginning of clinical signs and monitor the poisoning, revealing characteristic alterations that aid the diagnosis and facilitate the understanding of the poisoning pathophysiology.

Animal deaths were caused by heart failure characterized by arrhythmias, tachycardia/ventricular fibrillation, drop in cardiac output, left ventricular (LV) systolic dysfunction due to progressive decrease in LV ejection fraction (EF), decrease in fractional shortening (FS) of the LV, decreased aortic flow velocity and aortic flow gradient. These changes were evidenced on the electro and echocardiogram.

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