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Ligation of the left renal vein in epm1-wistar rats: functional and morphologic alterations in the kidneys, testes and suprarenal glands

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Objective: The ligation of the left renal vein (LLRV) in man is a controversial procedure in view of the risks of lesion to the renal parenchyma. With the objective of studying the morphologic and functional alterations caused by these lesions, we conducted experimental research with rats. **Material and Methods:** 64 male adult EPM1-WISTAR rats were used, divided into 8 groups - 4 for LLRV and four for control. Each LLRV group and corresponding control group were sacrificed progressively on the 7th, 15th, 30th and 60th day after the initial surgery. **Results:** We found morphofunctional alterations only in animals that underwent LLRV in the four periods of sacrifice. The proteinuria creatinine in serum, testosterone in serum and serum corticosterone in serum showed practically no alteration in relation to the normal values for rats. Statistically significant severe histological lesions were found in the kidneys and testes of the LLRV groups. Lesions in the suprarenal glands were also present in these groups, but no sufficient to demonstrate statistical significance. **Conclusion:** Based on these results we can conclude that the ligation of the left renal vein is a procedure of high risk in these animals.

Uniterms: Renal Veins. Ligation. Kidneys. Testes. Suprarenal glands.

INTRODUCTION

Frequently, the surgeon finds himself in a difficult situation in relation to normal human anatomy. Due to the syntropy of the elements of the human body, a good surgical approach to a certain organ or segment of the body can sometimes cause damage to another element with significant repercussions. The left renal vein (LRV) due to its syntropy with the abdominal aorta, may hamper an ideal approach to that artery.¹

As the LRV has many tributaries, many authors recommend its ligation or temporary section to facilitate the surgical procedure.²⁻²⁷ However, in the case of the right renal vein (RRV) which has no tributaries, its ligation will certainly result in severe renal lesion, and consequently this procedure is forbidden.²⁸⁻³² The presence of multiple renal veins is most frequent on the right, which is shorter, drains into the cava and has no relationship to the aorta. The LRV is normally unique, longer, and crosses transversally in front of the aorta before draining into the inferior vena cava and generally receives three tributaries.²⁸⁻⁵²

Many complications have been published after the ligation of the left renal vein (LLRV), including loss of the renal function requiring hemodialysis, retroperitoneal hemorrhage, increase in creatinine levels, congestion of the renal vein, renal necrosis, rupture of the left kidney, thus increasing the patient's morbidity.^{2, 9, 10, 11, 14, 34, 44, 53-70} To avoid renal complications⁷¹⁻⁷⁴ reanastomosis has been recommended

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as a routine after the section of the LRV. The blood flow to an organ decreases when the venous pressure nears the diastolic blood pressure.^{75,76} The LLRV enables the inversion of the venous flow and venous hypertension in the kidney, testes and suprarenal gland.^{8, 14, 21, 50, 60, 67, 77-89}

Baptista-Silva (1994)¹ in more than 200 surgeries of the aorta, never had the need to perform a ligation of LRV in elective surgery such as aneurysm emergencies or trauma to the aorta. In other words, when the approach to the aorta was difficult, he used other surgical techniques recommended by other authors,^{39, 53, 69, 71, 72, 87, 88, 90-103} to facilitate the dissection of the aorta and avoid a LLRV.

Many researchers, worried about the complications of LLRV and renal vein thrombosis, and interested in investigating varicocele developed experimental models in animals with similar clinical findings. Dogs and rats were used in these studies due to the similarity of their vascular anatomy, hemodynamic repercussion and immunology system to that of man. It was demonstrated that in dogs a LLRV increased 20 times the hilar renal venous pressure, reducing the renal arterial pressure, the thickening of the basal renal membrane, the high concentration of nephrotoxics antigen, proteinuria, hypoproteinemia, hypoalbuminemia, hypercholesterolemia, atrophy of the left kidney, arterial hypertension in the kidneys with renal congestion, reaction to immunocomplex with lesion of the right kidney and increase in mortality of animals.^{47, 75, 76, 83, 104-117}

Based on the current controversy in relation to renal lesion, and in the absence of literature on the incidence of lesion to the testes and suprarenal glands after the ligation of the left renal vein in man, we decided to research in rats the functional and histopathologic alterations caused by the LLRV in the kidneys, testes and suprarenal glands.

MATERIAL AND METHODS

Sixty four male adult EPM1-WISTAR rats were used, weighing around 264 to 469 grams, and were maintained under controlled light conditions from 6 A.M. to 6 P.M. and darkness from 6 P.M. to 6 A.M., at room temperature of $24 \pm 1^\circ\text{C}$ and free access to rat ration and water (1,118). The rats were randomized into 8 equal groups (8 animals each) - four groups for ligation of the left renal vein and four control groups. The groups were randomly chosen to be sacrificed on the 7th, 15th 30th and 60th day after initial surgery, always including one from the LIGATION group (L7, L15, L30, L60) and one from the CONTROL group (C7, C15, C30, C60).

Table 1
Distribution of the rats per group, type of procedure and sacrifice day

Groups	Nº of Rats	Type of Procedure	Sacrifice day
L7	8 {1 a 8 }	"ligation"	7th
C7	8 {9 a 16}	"control"	7th
L15	8 {17 a 24}	"ligation"	15th
C15	8 {25 a 32}	"control"	15th
L30	8 {33 a 40}	"ligation"	30th
C30	8 {41 a 48}	"control"	30th
L60	8 {49 a 56}	"ligation"	60th
C60	8 {57 a 64}	"control"	60th

The rats were identified from 1 to 64. "INITIAL" referred to the first day of surgery and "SACRIFICE" to the last day of the experience. (Table 1).

The animals were submitted to anesthesia by inhalation of ethyl ether. A medianus laparotomy of about 3 cm was performed, with deviation of the intestinal loops to the right, opening of the retroperitoneal and careful dissection of the structures and ligation of the left renal vein close to the inferior vena cava, with a 7-0 monofilament polypropylene thread, preserving the renal drainage to the suprarenal, testicular and renolumbar veins. Closure of the abdominal wall was performed using a 3-0 cotton thread. A laparotomy was performed in the control group, as well as dissection of the LRV; threading and withdrawal of a monofilament polypropylene 7-0 thread under the LRV was conducted without ligation. The animals were weighed before surgery and after sacrifice and were always sacrificed by decapitation between 10 and 12 hrs. The animals were beheaded and the blood collected from the neck vessels placed in heparinized tubes and sent for biochemical analysis. After the beheading and collecting of blood, the animals were submitted to xiphopubic laparotomy, assessment of the abdominal condition, accurate exam of the intra and retroperitoneal organs. The kidneys, testes and suprarenal glands were examined, withdrawn and weighed (humid weight), and submitted to histological analysis by light microscopy.

Statistical analysis of the creatinine, testosterone, serum corticosterone and proteinuria was obtained by means of an experimental plan with two factors as sacrifice date with four levels (7th, 15th, 30th and 60th day) and surgery with two levels, ligation and control, and their values by variance analysis for an hierarchical model.¹¹⁹ The results of percentual variation between the weight of the animals and their kidneys, testes and suprarenal gland

were analyzed using STUDENT *t* test¹²⁰; the histopathological results were analyzed using FISHER's exact test.¹²¹ The significant values were marked with an asterisk (*). The tests were performed at a 5% ($p < 0.05$) level of significance.

RESULTS

The examination of the abdominal wall of the rats showed good cicatrization and no infection was detected. All the rats of the ligation and control groups were submitted to an exam of the intraperitoneal cavity and no visceral abnormalities were found. The alterations observed in the retroperitoneum were related to the size and color of the kidneys and suprarenal glands and main veins and tributaries. No thrombosis of the renal vessels and tributaries was observed either in the ligation or control groups.

The left kidneys of ligations were swollen and had a violet coloring by day 7 and 15, but on day 30 they had reduced in size and were more cyanotic. On the 60th day the reduction in size was more apparent and the coloring was darker with the left kidney showing an increase in size. The ligations of the LRV and their tributaries were always dilated.

The testes of ligation groups, to the left, the venous network was dilated; on the 60th day the left testis was always smaller than the right one.

The suprarenal glands were too small for the observation of macroscopic differences between them. The suprarenal venous network to the left of the ligation was enlarged compared to the control. (Fig 1, Table 2)

The results of the proteinuria, serum creatinine, serum testosterone and serum corticosterone were statistically different in some periods of the controls but did not exceed the values of normality for rats.

HISTOPATHOLOGY CHANGES BY LIGHT MICROSCOPY

Kidneys

In the ligation groups, we found severe lesions due to ischemia, classified as atrophy of the parenchyma, papilla necrosis, lesion of the distal tubuli, severe ischemia, infarction and necrosis of the kidneys of the rats sacrificed

Table 2

Absolute results of WEIGHT: Body (initial and sacrifice), of the Kidneys, Testes and Suprarenal glands. BIOCHEMICAL EXAMS: Proteinuria, Serum Creatinine, Serum Testosterone, Serum Corticosterone. GROUPS: L = Ligation, C = Control; and 7, 15, 30 e 60 refer to the sacrifice day. MEAN and SD (Standard Deviation).

Groups	Weight (g)								Biochemical Exams			
	Body		Kidney		Testis		Suprarenal		creatinine	proteinuria	testosterone	corticosterone
	Initial	Sacrifice	Right	Left	Right	Left	Right	Left	mg/dl	mg/24h	ng/dl	µg/dl
L 7 Mean	289.88	280.00*	1.158	1.324	1.375	1.487	0.030	0.0340	500*	11.828*	87.125*	7.549*
SD	24.175	23.568	0.153	0.479	0.282	0.232	0.004	0.006	0.053	1.994	33.989	3.253
C 7 Mean	276.88	273.75	1.071	1.070	1.500	1.449	0.027	0.030	0.375	9.086	142.375	3.328
SD	19.628	21.218	0.101	0.099	0.074	0.094	0.007	0.008	0.046	1.540	45.074	2.246
L 15 Mean	290.00	294.50 *	1.234 *	1.455 *	1.413	1.399	0.028	0.028	0.488 *	12.103 *	126.375	8.231
SD	25.355	26.533	0.146	0.357	0.344	0.358	0.005	0.006	0.035	3.203	50.622	5.257
C 15 Mean	281.63	292.25	1.083	1.064	1.534	1.550	0.025	0.025	0.413	8.313	108.625	5.904
SD	28.948	29.688	0.150	0.149	0.122	0.126	0.005	0.006	0.064	1.8812	2.206	2.123
L 30 Mean	288.00	300.75	1.103	1.021	1.418	1.436	0.030*	0.031*	0.438	14.328*	115.000	10.129
SD	20.227	19.241	0.116	0.214	0.226	0.188	0.006	0.008	0.052	4.0123	33.785	4.583
C 30 Mean	317.00	326.50	1.096	1.122	1.523	1.556	0.025	0.024	0.400	8.840	129.375	8.280
SD	11.225	12.306	0.083	0.108	0.093	0.077	0.003	0.004	0.093	1.5841	10.225	4.615
L 60 Mean	315.50	331.88*	1.441*	0.576*	1.459	1.243*	0.026	0.027	0.363*	14.283*	117.000	7.650
SD	34.422	44.315	0.216	0.296	0.374	0.514	0.004	0.002	0.052	3.7024	46.522	1.834
C 60 Mean	309.75	338.75	1.124	1.109	1.638	1.678	0.024	0.023	0.288	10.725	144.750	7.676
SD	33.247	37.583	0.152	0.112	0.131	0.154	0.006	0.004	0.099	3.5445	50.821	1.927

* $p < 0,05$

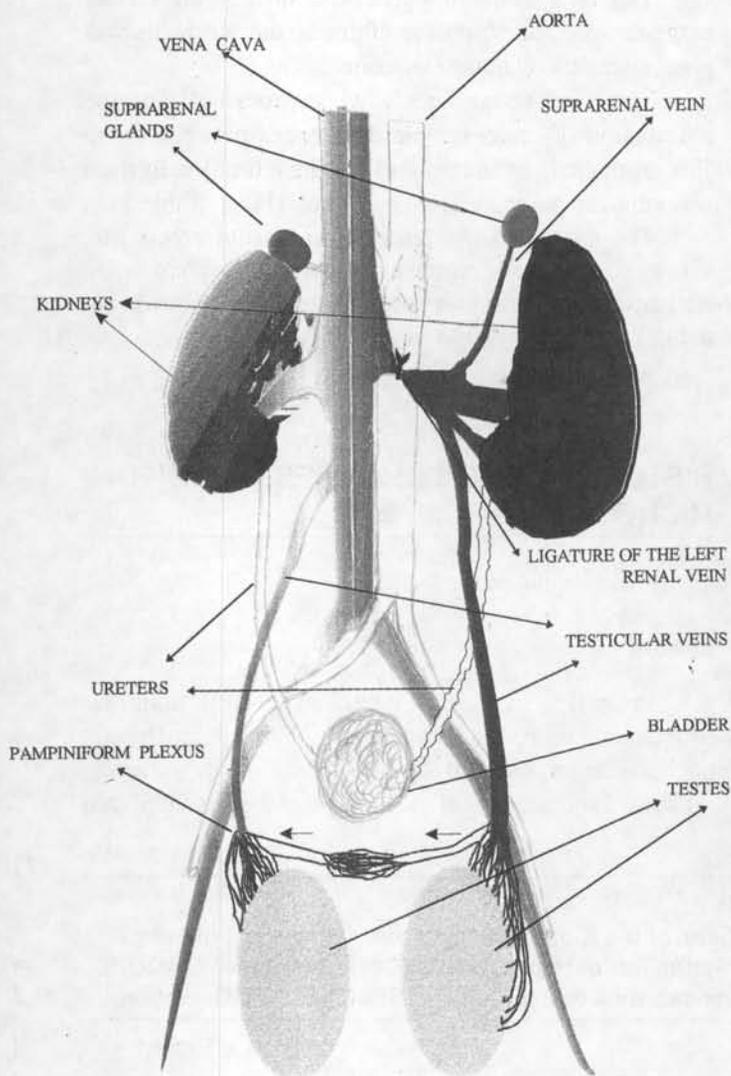


Figure 1 - Ligature of the left renal vein, seven days after the initial operation. It is shown on the left side the dilatation of following veins: renal, suprarenal, testicular, of the pampiniform plexus, of the ureter and increase of the kidney.

on the 7th, 15th, 30th and 60th days after the initial operation. They were more severe to the left than to the right. Statistical differences were only observed on the left side, in the ligation groups, compared with the control groups in the 7th, 15th, 30th and 60th day. $p < 0,05$ (Table 3, Fig. 2)

Testes

In the ligation groups, we found severe lesions due to ischemia, classified as atrophy, necrosis, diffuse fibrosis with calcification of the testes of the rats sacrificed on the 7th, 15th, 30th and 60th days after the initial operation. Significant statistical bilateral differences were only

observed on the 30th day and to the left on the 60th day, in relation to the control group, and were more severe to the left than to the right. $p < 0,05$ (Table 3, Fig. 3)

Suprarenal Glands

In the ligation groups, we found severe lesions due to ischemia, classified as cortical atrophy, medullar and cortical necrosis of the suprarenal glands of the rats sacrificed on the 7th, 15th, 30th and 60th days after the initial operation, but without statistical differences in relation to the control group. (Table 3) $p > 0,05$.

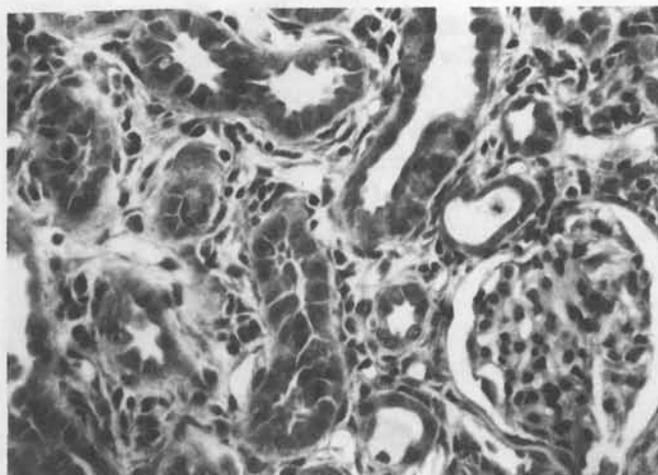


Figure 2 - Left kidney of rat # 55 of the ligation group, showing alterations of the ischemic type, diffuse, with retracted, enlarged mesangial matrix and tubuli with atrophy signs, surrounded by interstitium with fibrosis and rare lymphocyte infiltrates. (HE - 126x)

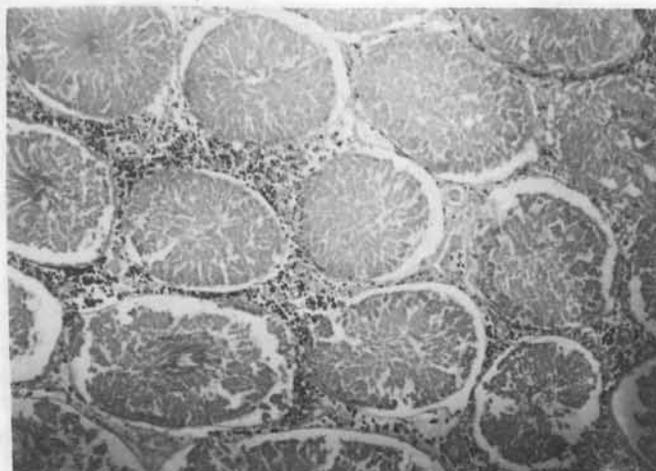


Figure 3 - Left testis of rat # 38 of the ligation group, showing diffuse coagulation necrosis. Interstitial inflammatory exudate is also present. (HE - 79x)

Table 3
Results of the histopathology of the kidneys, testes, and suprarenal glands. GROUPS: L = Ligature, C = Control; and 7, 15, 30 e 60 refer to the sacrifice day. (* $p < 0.05$ by Fisher exact test)

Groups	Histopathology	Kidneys		Testes		Suprarenal Glands	
		Right	Left	Right	Left	Right	Left
L 7	Normal	6	3	6	8	6	7
	Pathologic	2	5*	2	0	2	1
C 7	Normal	8	8	8	8	8	8
	Pathologic	0	0	0	0	0	0
L 15	Normal	7	0	5	5	7	5
	Pathologic	1	8*	3	3	1	3
C 15	Normal	8	8	8	8	8	8
	Pathologic	0	0	0	0	0	0
L 30	Normal	7	2	3	2	6	6
	Pathologic	1	6*	5*	6*	2	2
C 30	Normal	8	8	8	8	8	8
	Pathologic	0	0	0	0	0	0
L 60	Normal	8	0	6	4	8	6
	Pathologic	0	8*	2	4*	0	2
C 60	Normal	8	8	8	8	8	8
	Pathologic	0	0	0	0	0	0

DISCUSSION

In this study we found biochemical, functional and histological alterations as a result of the ligation of the left renal vein. Confirming previous studies on the subject, dilation of the left renal vein and its tributaries was found only in the ligation groups, in all periods.^{61,75,86,106,115,122,123}

In the LLRV animals, we found a significant variation of body weight (less weight increase compared to the control group) on the 7th, 15th and 60th day after the initial operation. However, this variation was not present when only a stenosis of the LRV was performed.⁸⁶

At the end of the experiment hypertrophy was found in the right kidney, and atrophy in the left. These alterations showed that the right kidney increased in size because of overload, and the left kidney lost weight due to severe histological injuries caused by ischemia due to an extended venous stasis. These results are the same reported by other authors.^{2,61,75,124-127} In dogs submitted to ligation of the left renal vein there was a decrease of blood flow on the left as a consequence of venous stasis⁷⁵ and in man the variation of the pressure in the hilar renal venous

stump was dependent on the flow through the tributaries of the LRV with the pressure above 60 cm of water in the hilar renal venous stump possibly leading to a decrease of the glomerular filtration due to the decrease of the renal arterial flow.^{17,54} We found an increase in creatinine serum and proteinuria in the ligature groups, although these values were practically normal. Light proteinuria was observed in rats submitted to LLRV. Massive proteinuria, such as that observed in nephrotic syndrome, was only possible in severe renal injury.¹²⁴ It is difficult to obtain massive proteinuria in an experimental study.¹²⁸ Failure to produce massive proteinuria from an affected kidney or nephrotic syndrome may be partially due to the insufficient increase of the renal venous pressure. Significant proteinuria occurred only in a dog with high renal venous pressure (30 cm of saline).¹²⁶ In acute experiments Wegria et al.¹²⁹ found that protein was not present in the urine when the pressure in the left renal vein of the dog was of less than 24,5 cm of water. A pressure between 24,5 and 42 cm of water in the left kidney excreted protein in 17 of the 27 animals under study. In pressures above 42 cm of water, proteinuria was practically always present.¹²⁹ In dogs the pressure of the left renal vein after ligation varied between 14 and 30 cm of water, and was not associated with significant proteinuria from either the affected or the control kidney.¹²⁶ In the histological analysis we observed that the kidneys had a severe lesion due to diffuse ischemia, atrophy of the parenchyma, necrosis of papilla, infarction and fibrosis in all groups of ligation especially in the left kidney, with the biochemical alterations (serum creatinine and proteinuria) remaining near the limits of normality for rats. Histological alterations were related to an increase in protein droplets in the tubular cells, leukocyte margination in the glomerular capillaries, and thickening of the glomerular membrane.¹²⁶ This showed that in the case of LLRV biochemical analysis alone is not sufficient to uncover a renal lesion as already reported in medical literature.^{61,75,114,124,125,127,130} Our results showed that renal lesions were predominantly on the left side in all periods. Nevertheless, lesions on the right side were present only in the ligature groups suggesting that there are other factors contributing to a lesion in the right kidney. This could possibly be due to an immunocomplex reaction as a result of an injury to the left kidney, similar to that described by other investigators.^{75,110}

In the testes, atrophy was found on the left on the 60th day, a finding already described in experimental stenosis of LRV.⁸⁶ There was practically no variation of the serum testosterone level throughout the periods. Similar alteration of the serum testosterone level was reported by other authors in relation to patients with varicocele and also in animals

with stenosis of LRV. These authors have also found an increase of luteinizing hormones, follicle-stimulating, prolactin, oligospermia and zoospermia.^{104,107,108,117,131} The histological analysis of our study showed a severe bilateral testicular lesion in three of the four groups of ligation, more pronounced to the left on the 30th and 60th day. Other researchers found bilateral testicular lesions and they considered that these could be induced by immunocomplex reaction.^{86,132-137} Other studies must be conducted to further investigate testicular injuries caused by varicocele,¹³⁸ but it has been suggested that a defect in the testicular energetic metabolism caused by varicocele may be associated to the worsening of spermatogenesis in rats.¹³⁹

In the suprarenal glands we found bilateral histological lesions in most periods, but with no statistical

difference, although we could find nothing in medical literature concerning suprarenal gland lesion provoked by varicocele or by LLRV. Other authors reported suprarenal gland lesions such as hemorrhage and necrosis, caused by stress, operation or post thrombosis of the inferior vena cava or of LRV in the newborn.^{64,77,78,84}

We can report that the ligation of the left renal vein close to the inferior vena cava in male adult EPM1-WISTAR rats leads to a severe bilateral histological lesion in the kidneys, testes especially on the left side. The results of the biochemical analysis were not sufficient to demonstrate functional alterations. Histopathological analysis was considered the best test for assessment of lesions caused by LLRV.

RESUMO

Objetivo: A ligadura da veia renal esquerda é conduta discutida na literatura pelos riscos de lesão do perênquima renal. Com o objetivo de estudar morfológica e funcionalmente essas lesões, realizamos trabalho experimental em ratos. **Material e Método:** foram utilizados 64 ratos machos adultos da linhagem EPM1-WISTAR, divididos em oito grupos. Em quatro grupos efetuou-se a ligadura da veia renal esquerda enquanto os outros quatro serviram de "grupo controle". Cada grupo com veia ligada e respectivo "grupo controle" foram sacrificados sucessivamente no sétimo, 15º, 30º, e 60º dia após a operação inicial. **Resultados:** encontramos alterações morfo-funcionais apenas nos ratos submetidos a ligadura e em todos períodos de sacrifício. Os resultados de proteinúria e as dosagens plasmáticas de creatina, testosterona e corticosterona pouco se alteraram em relação aos valores normais. Lesões histológicas graves e estatisticamente significante apareceram nos rins e testículo nos grupos submetidos à "ligadura da veia renal esquerda", embora fossem observados também nas glândulas supra-renais, porém sem significado estatístico. **Conclusão:** com base nestes resultados, concluímos que a ligadura da veia renal esquerda nestes animais é de alto risco.

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