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Clinical Reports

Blood transfusion in a young *Myrmecophaga tridactyla* (Pilosa, Myrmecophagidae): case report

Transfusão sanguínea em *Myrmecophaga tridactyla* filhote (Pilosa, Myrmecophagidae): relato de caso

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ABSTRACT

The present study aims to report a transfusion therapy in a juvenile *Myrmecophaga tridactyla*. The patient had a chronic nonregenerative anemia associated with underdevelopment. This therapy was considered after a severe worsening of the clinical condition and laboratorial tests, in which severe anemia was confirmed, with no signs of recovery. The blood used was donated by a clinically healthy adult male of the same species, raised in captivity. The procedure was performed according to medical practice for small animals, since veterinary medicine reports described for xenarthrans are scarce. The result was satisfactory, with absence of late transfusion reactions and an improvement of the patient's overall condition, which demonstrates the possibility of using blood transfusion in anteaters as a therapeutic option.

Palavras-chave:
Xenarthra
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RESUMO

O presente trabalho tem como objetivo relatar a realização de terapia transfusional em um exemplar filhote de *Myrmecophaga tridactyla*. O paciente apresentava um quadro crônico de anemia arregenerativa associada a um subdesenvolvimento. A terapia foi definida após piora severa do quadro clínico e dos exames laboratoriais, nos quais observou-se anemia severa, sem sinal de regeneração. O sangue utilizado foi doado por um adulto da mesma espécie, macho, clinicamente hígido, de cativeiro. O procedimento foi realizado de acordo com o prescrito pela clínica médica de pequenos animais, visto que, os relatos descritos na medicina de xenarthras são escassos. O resultado se mostrou satisfatório, com ausência de reações transfusionais tardias e melhora do quadro geral do paciente, o que demonstra a possibilidade da utilização de transfusão sanguínea em tamanduás como opção terapêutica.

INTRODUCTION

The giant anteater, *Myrmecophaga tridactyla* (Linnaeus, 1758) is a mammal that belongs to the superorder Xenarthra, order Pilosa, being allocated in the Myrmecophagidae family. It is distributed throughout South and Central America, being particularly abundant in the Cerrado biome, where it suffers due to fires (particularly those related to slash-and-burn agriculture), and degradation of its habitat for agricultural production and fragmentation of its territory by roadways, being thus classified as "Vulnerable" by the International Union for Conservation of Nature (IUCN, 2014).

Anteaters do not exhibit permanent socialization, with animals forming pairs only during the reproductive period, and with an intense parental relationship between a mother and its cub, which remain together during the first nine months of the cub's life. In the cases

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in which the mothers die, orphans present a markedly low chance of survival. A considerable number of these orphaned cubs arrive annually in centers of wildlife rehabilitation, requiring intensive care, specifically directed to this species (MIRANDA, 2012).

Regarding the most common clinical occurrences related to captive specimens of xenarthrans, it is possible to mention traumas by barbed wire, fights and improper manipulation, while cases of collisions with automotive vehicles and injuries caused by predators are the most common occurrences involving free living animals (MIRANDA, 2012). In most of the aforementioned treatments, the animal arrives at rehabilitation centers in a critical health condition and may exhibit severe anemia, caused by factors such as acute blood loss and sepsis. This condition may rapidly progress to death if adequate emergency and supportive therapy are not provided, and transfusion therapy is necessary in some cases (PINCELI, 2010).

Raines and Stormes (2015) reported a case of blood transfusion in a specimen of collared anteater (*Tamandua tetradactyla*), treated at the Dallas Zoo Hospital. The animal underwent a surgical procedure for amputation of part of the tail due to self-mutilation and, after surgery, was reported as weak, ataxic, lethargic and with a 9% hematocrit count, after which transfusion therapy was indicated. The homologous transfusion was successful and the normalization of the hematocrit was progressive. In this case, the authors suggested, as a probable cause of the acute anemia, the excessive consumption of red cells due to the sepsis that followed the necrosis of the tail tip.

Considering the overall lack of information about emergency procedures in Xenarthran medicine, the present work aims to report a homologous blood transfusion in a specimen of *Myrmecophaga tridactyla*, being the first case report for this species, as far as the authors are aware of.

CASE REPORT

A male specimen of *Myrmecophaga tridactyla*, weighing 4.8 kg, was received in the Wild Animals Department of the Veterinary Hospital of University of Brasília (UnB), sent by the Wild Animals Triage Center (CETAS-DF). The animal had several wounds on its body, and the suspicion was that it had been attacked by another animal.

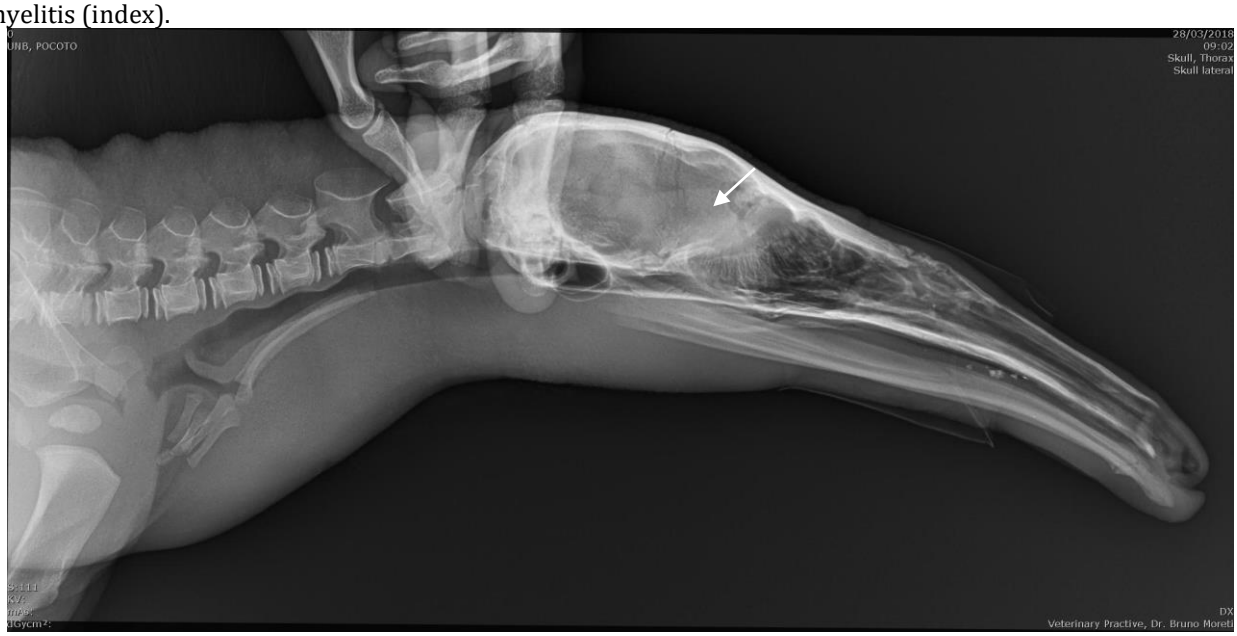
Initially, therapy was directed to the treatment of the wounds and stabilization of the patient's clinical condition, which was diagnosed with neonatal triad - dehydration, hypoglycemia and hypothermia. Subcutaneous fluid therapy was administered, initially calculating volume for a dehydration rate of 10%, followed by a 50% glucose solution orally administered, at a dose of 1 $mg.kg^{-1}$, with forced feeding via esophageal catheter, in addition to heating, performed by thermal bags and space heaters.

For analgesia, meloxicam (Maxicam®, Ouro Fino, Cravinhos, SP) was used for three days, administered intramuscularly (IM) once a day (SID), at a dose of 0,25 $mg.kg^{-1}$. Antibiotic therapy was established with enrofloxacin (Kinetomax 10% ®, Bayer Animal Health, Ribeirão Preto, SP) at a dose of 5 $mg.kg^{-1}$ IM every 96 hours, with a total of three applications associated with a single administration of sulfadoxine with trimethoprim (Borgal®, MSD Saúde Animal, São Paulo, SP) at a dose of 20 $mg.kg^{-1}$ IM.

After resolution of the initial clinical condition, the animal was maintained in the department to monitor the progression of its clinical condition, which was not satisfactory throughout the hospitalization period. The animal had difficulties in adapting to the diet, exhibiting low gains of body mass and bone development. After clinical, ultrasonographic, radiographic and laboratorial evaluations (including parasitological and clinical pathology analyses), the pup was found to have considerable hepatic and renal alterations, along with the recurrent detection of parasitism by *Ancylostoma* spp. and coccidia. Blood exams are elicited in Table 1. The antiparasitic therapy administered was febendazole (Panacur® 10%, MSD Saúde Animal, São Paulo, SP) at a dose of 50 $mg.Kg^{-1}$, orally (VO), SID, for five days, associated with sulfamethoxazole with trimethoprim (Bactrim®, Roche Chemicals and Pharmaceuticals, Jaguaré, SP) at a dose of 20 $mg.Kg^{-1}$, VO, twice a day (BID) for five days, and silymarin, a natural hepatic protector at the dose of 30 mg.Kg-1, VO, SID.

Although the antibiotic therapy was initially instituted for the treatment of wounds, the animal exhibited symptoms of osteomyelitis in nasal bone, which was confirmed by radiographic examination (Figure 1). We opted for surgical debridement, and a trepanation was performed to remove contaminated bone fragments, to collect material for bacterial culture and subsequent antibiogram, and to wash the affected area with a 2% iodopovidone solution diluted in saline. The antibiogram indicated bacterial resistance to previously used antibiotics (sulfonamides, fluoroquinolones and penicillin), indicating sensitivity only to cephalosporins. Ceftriaxone (Triaxon®, Teuto, Anapolis, GO) was then used at a dose of 25 $mg.kg^{-1}$, IM, BID, for 15 days.

Figure 1. Skull's left-lateral radiographic projection of a young *Myrmecophaga tridactyla* exhibiting nasal bone osteomyelitis (index).



Source: Wild Animals Departament of the Veterinary Hospital of University of Brasília (UnB)

In addition to osteomyelitis, the animal also had persistent anemia, even with support and mineral and vitamin supplementation treatment, with Hemolitan® at a dose of 1 drop.kg^{-1} (Vetnil, São Paulo, SP) and Ferrodex® (Fabiani Animal Health, São Paulo, SP) at a dose of 10 mg.Kg^{-1}, IM, every 7 days, with three applications. The anemia showed minimal to no signs of recovery (Table 1), with a progressive regression of the general clinical condition and presence of lethargy, exercise intolerance, and hyporexia. After 8 months (07/18/2018), the animal was reported as apathetic, and a physical examination reported moderate dehydration (7%), submandibular edema, fur loss, weight loss and hyporexia. Blood samples were collected for a haemogram, which demonstrated a case of severe nonregenerative anemia (Table 2).

The blood transfusion procedure was performed using data from domestic animal literature (GONÇALVES, 2012), due to the lack of information about transfusion therapies in species of the order Pilosa. The importance of new studies is emphasized, mainly due to the morphophysiological differences between carnivores and xenarthrans. Faced with the results of severe anemia, an exchange blood transfusion was chosen.

A calculation of 20 mL.kg^{-1} of blood for transfusion was performed (GONÇALVES, 2012), resulting in one in 80 mL of total volume. The donor was a healthy, 40 kg adult male *Myrmecophaga tridactyla*, belonging to the Zoological Foundation of Brasilia. The donor animal was submitted to chemical containment with a combination of ketamine at a dose of 10 mg.kg^{-1} and xylazine at a dose of 0.5 mg.kg^{-1} in the same syringe, IM route. The donor anteater showed good sedation results, which enabled the performing of the entire collection procedure without the need for increased anesthetic dosage or inhalation anesthesia. It was not possible to perform the pre-transfusional haemogram of the donor, but during blood collection, a sample was sent for laboratory examination (Table 2). The collected blood was stored in a 500 mL capacity blood collection bag containing citric acid, sodium citrate, sodium phosphate, dextrose and adenine (CDPA-1). One mL of anticoagulant was calculated for each 7.1 mL of blood, and 11.2 mL of anticoagulant were then used for 80 mL of whole blood. The excess volume was removed from the bag and discarded. By the end of the procedure, the donor woke up from anesthesia with no complications, same as during the donation process. The animal remained healthy for following months after the procedure, with no clinical or behavioral changes.

The blood to be transfused was transported to University of Brasília in styrofoam box, being refrigerated with recyclable ice, with an interval of approximately 3 hours between blood collection and initiation of the transfusion procedure. Immediately after the arrival of the material in laboratory of Veterinarian Pathology Clinical (UnB), a blood compatibility test was performed with a cross-matching test, with the donor being deemed compatible with the recipient.

For the blood transfusion, the juvenile was chemically restrained with the association of ketamine (Ketodex®, Venco Saúde Animal, Londrina, PR) 8 mg.kg^{-1} and 0.2 mg.kg^{-1} of midazolam (Dormire®, Cristália, Itapira, SP), IM, which promoted intense sedation in the animal, allowing for the manipulation and the beginning of monitoring within 10 minutes of the application. A 22-gauge peripheral IV catheter was placed in the cephalic vein as an emergency route and as a transfusional route. Oxygen therapy was performed by facial mask in the initial 40 minutes, with a flow rate of 4 L/minute. One hour after the beginning of sedation, the animal started

responding to tactile and auditory stimuli, and for that reason 1 mg.kg^{-1} of xylazine was applied, half of the dose being taken via the IM route and the other half subcutaneously (SC), simultaneously.

Table 1. Haemograms of the juvenile *Myrmecophaga tridactyla* performed during the hospitalization period before the transfusion procedure.

Haemogram	First clinical evaluation (05.12)	After clinically stabled (24.01)	Therapy monitoring (16.02)	Therapy monitoring (07.03)	Therapy monitoring (20.03)	Therapy monitoring (10.04)	Therapy monitoring (27.04)	Reference values[1]
HCT (%)	28	25	29	28	29	27	26	38,08 ± 5,93
RBC (x 10^6/µL)	2,31	1,56	2,24	2,07	2,19	1,67	1,65	2,07 ± 0,40
Hb (g/dL)	5,6	7,2	9,6	9,2	9,7	7,8	6,7	11,33 ± 2,15
MCV (fl)	121	160	129	135	132	161	157	186,52 ± 21,72
MCHC (%)	20	29	33	33	33	29	26	29,68 ± 2,56
Absolute (x10^3 /µL)								
WBC	10,1	6,2	3,6	6,4	3,3	4,7	6,3	7,8 ± 2,2
Neutrophils	1010	3658	1512	4992	1716	3780	5922	5779 ± 2180
Lymphocytes	5454	1922	1548	1216	1318	611	315	1483 ± 508
Monocytes	1111	124	72	64	198	94	-	155 ± 92
Relative (%)								
Neutrophils	10	59	42	78	52	80	94	71,50 ± 10,34
Lymphocytes	54	31	43	19	42	13	5	18,50± 8,25
Monocytes	11	2	2	1	6	02	-	3,33 ± 2,57
TP (g/dl)	6,2	5,4	6,4	5,8	6,0	5.8	4,6	6,23 ± 0,49
Platelets	-	236000	254000	292000	287000	216000	210000	123458 ± 31362
Reticulocytes (%)	-		-					-
Observation	Platelet aggregates, chistocytes, codocytes, acanthocytes, vacuolated monocytes, toxic neutrophils, cytoplasmic granulation, Dohle corpuscle; reactive lymphocytes	Reactive lymphocytes, codocytes	Reactive lymphocytes	activated platelets, platelet aggregates	Anisocytosis, microcytes, schistocytes	Anisocytosis, polychromatophils, acanthocytes and rare schistocytes.	Poikilocytes	-

[1] OLIVEIRA et al. (2017)

Table 2. Haemograms of the juvenile *Myrmecophaga tridactyla* before and after the blood transfusion procedure, and the donor's haemogram.

Haemogram	Pre-transfusion (18.07)	Post-transfusion (24.07)	Post-transfusion (02.08)	Donor	Reference values[1]
HCT (%)	10	18	11	36	38,08 ± 5,93
RBC (x 10^6/µL)	0,68	0,97	0,93	2,16	2,07 ± 0,40
Hb (g/dL)	3,3	4,3	3,6	11,3	11,33 ± 2,15
MCV (fl)	147	185	120	159	186,52 ± 21,72
MCHC (%)	33	23	33	33	29,68 ± 2,56
Absolute (x10^3 /µL)					
WBC	6,0	3,8	3,5	6,6	7,8 ± 2,2
Neutrophils	4360	3116	2940	4554	5779 ± 2180
Lymphocytes	660	608	525	1618	1483 ± 508
Monocytes	480	76	35	148	155 ± 92
Relative (%)					
Neutrophils	81	82	84	69	71,50 ± 10,34
Lymphocytes	11	16	15	23	18,50± 8,25
Monocytes	8	2	1	3	3,33 ± 2,57
TP (g/dl)	4,2	4,2	4,4	6,8	6,23 ± 0,49
Platelets	400000	208000	134000	150000	123458 ± 31362
Reticulocytes (%)	-	2,1 (35890)	-		-
Observation	Anisocytosis (+), polychromasia (+)	Anisocytosis (+), polychromasia (+)	Anisocytosis (+) Reactive lymphocytes Platelet aggregate	Hemolyzed serum	-

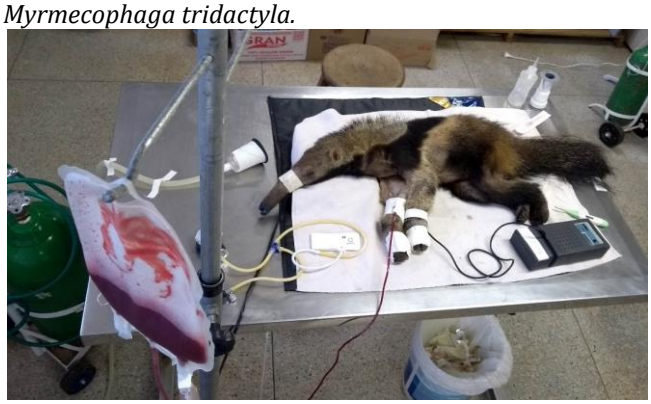
[1] OLIVEIRA et al. (2017)

The transfusion was initiated after the blood was warmed up in a water bath, in a container with no contact with the water, at a temperature of 36 °C and coupled to a double chamber transfusion set. The blood was taken out of the water when it reached the temperature of 34ºC. The infusion was performed at the rate of 0,25 ml.kg^{-1}.h^{-1} for the first 30 minutes and then increased to 5 ml.kg^{-1}.h^{-1} up to 7 ml.kg^{-1}.h^{-1} after 1 hour and 10 minutes. After 45 minutes of transfusion, the animal displayed a mild edema in the periocular region, and 5 mg.kg^{-1} hydrocortisone sodium succinate (Ariscorten®, Blau Farmacêutica, São Paulo, SP) were administered intravenously. Heart rate, respiratory rate, temperature, and blood pressure (systolic arterial pressure) were measured every five minutes throughout the whole procedure, with mean and standard deviation values being: HR 102,66 (± 13,30), RR 6,58 (±2,41), SAP 93,44 (± 2,9), and TºC 34,75 (± 0,58). Vital signs were measured by stethoscope, visual observation of thoracic movements and by the use of doppler ultrasound with a number 3 arm cuff, respectively (Figure 2).

The total time of the procedure was approximately 3 hours. By the end of it, 40 mL of warmed Lactate Ringer were administered via SC. The animal recovered smoothly but steadily and walked spontaneously 15 minutes after the end of the transfusion, without ataxia.

As supportive therapy, we performed the oral administration of a vitamin-mineral supplement (Hemolitan®, Vetnil, São Paulo, SP) 1 drop.kg^{-1}, BID, for 21 days, and of thymomodulin immunostimulant (Leucogen®, Aché Laboratorios Farmacêuticos SA, Guarulhos, SP), in the volume of 5 mL, VO, BID, for the same period.

Figure 2. Blood transfusion procedure in a young *Myrmecophaga tridactyla.*



Source: personal archive, 2020.

The animal remained under constant observation for a 72 hours' post-transfusion period. It did not present any clinical signs compatible with late transfusion reactions. One week after the transfusion, a blood collection was

performed, which indicated an increase in hematocrit, hemoglobin and red blood cell count (Table 2).

A second haemogram was performed 12 days after the transfusion (Table 2), showing a new decrease in red blood cells and haematimetric values. Clinical support was retained and a second transfusion was rethought, but the clinical condition of the specimen worsened considerably, and death occurred.

The animal was referred to the Laboratory of Veterinary Pathology of UnB, and a post mortem examination was carried out, which revealed alterations in the hemolymphopoetic system, with marked medular erythroid hypoplasia compatible with aplastic anemia of the bone marrow.

DISCUSSION

Blood transfusion is an emergency treatment that aims at temporary, effective and safe replacement of blood components. Transfusion therapy aims to meet basic needs to maintain the animal's life, so there is time to take specific measures against the primary cause of anemia (COTTER, 1991; PINCELLI, 2010). In order to decrease risks associated with transfusion, alternative therapy can be made, such as the use of parenteral iron. Although controversial, parenteral iron can improve RBC and decrease transfusion requirements (SUFFREDINI *et al*., 2017). However, it did not occur with this patient, thus being necessary a further whole blood transfusion.

The main indications for the transfusion are massive blood loss as a consequence of hemorrhage, hemolysis or non-regenerative anemia. The transfusion should not be based just on numerical values, and it is also necessary to make a complete evaluation of the patient's clinical condition. Clinical parameters that may indicate the need for transfusion are non-specific and may involve pale mucous membranes, hypothermia, tachycardia, tachypnea, and increased capillary refill time. These should be associated with parameters from laboratorial tests and patient history, and only then should a decision regarding the need for the procedure to be made (COTTER, 1991; PINCELLI 2010). The severe anemia exhibited in the present case justified the need for a transfusion, given the nonregenerative nature of the condition, nonresponsiveness to iron therapy, and the low hematological values (WEISS, 2003; SUFFREDINI *et al*., 2017).

Some authors cite the need for blood transfusion in small animals when hematocrit values are lower than 20%, in order to avoid damage due to hypoxia in vital organs (COUTO, 2003; GONÇALVES, 2012). Gonçalves (2012) points out that hemoglobin evaluation is a more reliable parameter, so that values below 7g.dL^{-1} would justify the need for transfusion of whole blood or packed red blood cells. Both conditions occurred in the patient, corroborating the need for transfusion therapy after parenteral iron therapy.

A gross estimate of the total amount of blood to be transfused is 10 to 20 mg.kg^{-1} of the recipient's weight, resulting in an average increase of 10% of the patient's hematocrit (GONÇALVES, 2012). In the present study, the same calculation was used, resulting in a final volume of 80 mL of blood to be transfused. Raines (2015), in a blood transfusion performed in a collared anteater, cites the use of the formula applied to domestic animals, from the desired hematocrit (%), multiplied by twice the body weight (kg). However, the author reported difficulty in obtaining the total amount of blood required, considering the present risk due to the low weight of the donor. Other authors suggest that 1 to 2 mg.Kg^{-1} of blood transfusion elevate the hematocrit by 1%, assuming the donor hematocrit is equal to 40% for dogs and 30% for cats (KRISTENSEN & FELDMAN, 1995). The hematocrit of the donor of the present report was 36%, falling within the average values stipulated for the domestic species. The transfusion rate was also based on small animal practice. The first rate administered was 0,25 ml.kg^{-1}.h^{-1} for the first 30 minutes to check any significant reaction, and then increased up to 7 ml.kg^{-1}.h^{-1}, based on previous data published ranging from 5 to 10 ml.kg^{-1}.h^{-1} in dogs and cats (GODINHO-CUNHA *et al*., 2011).

The use of the blood compatibility test (cross-reaction) was interesting in the context of the immediate success of the transfusion. The use of blood from a compatible donor maximizes the half-life of the transfused red blood cells and minimizes the occurrence of transfusion reactions (LACERDA et al., 2011). Despite the lack of literature on erythrocyte half-life in xenarthrans, it is known that the half-life of transfused cells is inferior to that of autologous red blood cells. In carnivores, for example, the average duration of red blood cells in the circulation is 110 days in canids and 68 days in felines, whereas transfused red blood cells it is 21 days in dogs and 55 days in cats (HARVEY, 2001). In the present study, the patient's hematocrit indicated a 15-day post-transfusion decrease, which may suggest that the half-life of giant anteater red blood cells is also lower in transfused cells, when compared to autologous cells.

Another essential factor during blood transfusion is constant patient monitoring. Parameters such as temperature, respiratory rate and heart rate should be checked every five minutes (TYLEY & SMITH, 2000). All of these parameters were measured, with the addition of a noninvasive blood pressure measurement, which would aid in the early detection of acute transfusion reactions. In the case of detection of changes such as temperature increases, vomiting, hemolysis, electrolytic disturbances, circulatory overload and urticaria, the transfusion should be immediately interrupted, with the necessity of fluid and glucocorticoids infusion (RAINES, 2015; GONÇALVES, 2012). Although the animal was under mild sedation in the present case, none of the aforementioned alterations were observed.

Only a slight eyelid edema was observed, and hydrocortisone and fluid therapy were administered

postoperatively, which reversed the condition without major complications. Reactions may occur during or after transfusion therapy, being classified as either acute or late, and as resulting from an immune reaction or not. There are several types of transfusion reactions reported and, in small animals, the most common are hemolysis (acute and late) and leukocyte and platelet hypersensitivity (LACERDA, 2008; PRADO, 2011). Considering that the anteater can be seen as phylogenetically related to carnivores, it is expected that there will also be potential complications in this species if all recommended precautions are not taken when performing an exchange blood transfusion (RAINES, 2015). However, further studies on transfusion reactions and on the half-life of red blood cells in xenarthrans should be performed.

The clinical improvement after the first 24 hours and the laboratory parameters observed in the first post-transfusion haemogram can be considered satisfactory, and although an immediate post-transfusion haemogram was not performed, it is believed that the hematocrit reached approximately 20%, as was initially intended. The clinical and laboratory involution showed by the patient can be justified by the nonregenerative aspect of the anemia, due to a hematopoietic disorder, of chronic course, by erythroid medullary hypoplasia, diagnosed in the postmortem examination.

The prognosis for the patient diagnosed with medullar aplasia is directly related to the cause of the disease, and to the course of the disorder: acute or chronic. Chronic spinal aplasia presents an unfavorable prognosis, since the great majority of cases are irreversible, being unresponsive to the established treatments (WEISS, 2003). The possible causes of medullary aplasia diagnosed in the present case may be associated with the chronically undernourished state of the cub, to the difficulty of adaptation to the liquid diet and the prolonged antibiotic-based therapy, among other drugs. During the maintenance of the patient in the sector, therapies based on sulfadiazine/trimethoprim, sulfamethoxazole, cephalosporins and antiparasitics, such as febendazole, were administered. These drugs are indicated in the literature as potential causes of medullary aplasia, due to the toxicity induced by them (WEISS, 2003; MORAES, 2010).

CONCLUSIONS

The transfusion therapy performed in the present case presented a satisfactory result, evidenced by the absence of late transfusion reactions, maintenance of the patient's hematocrit value after one week, and overall improvement of the clinical condition. Considering the benefits of using transfusion therapy, especially in emergency situations, the importance of further descriptions and protocol studies in members of the Mymercophagidae family is hereby reiterated.

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