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Spontaneous and experimental poisoning of cattle by *Palicourea aeneofusca* in the region of Pernambuco and induction of conditioned food aversion

Intoxicação espontânea e experimental por *Palicourea aeneofusca* em bovinos, no agreste de Pernambuco, e indução de aversão alimentar condicionada

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ABSTRACT

*The objective of this study was to describe the epidemiological, clinical, and pathological aspects of **Palicourea aeneofusca** poisoning in cattle in the region of Pernambuco, Brazil and to determine if it is possible to induce food aversion by **P. aeneofusca** poisoning in cattle raised under extensive management conditions. To determine the occurrence of poisoning, 30 properties were visited in five municipalities of the region of Pernambuco. Three outbreaks of poisoning of cattle were monitored. To induce conditioned food aversion by the consumption of **P. aeneofusca**, 12 animals were randomly distributed into two groups of six animals each. Cattle were weighed and received green **P. aeneofusca** leaves in their trough at a dose of 35mg kg^{-1} body weight for spontaneous consumption. The control group (CG) animals received water (1ml kg^{-1} body weight) via a feeding tube after the first ingestion of the plant, while the other animals, constituting the aversion test group (ATG), underwent induced aversion with lithium chloride (LiCl - 175mg kg^{-1} body weight) via a feeding tube. For the ATG cattle, the aversion to **P. aeneofusca** induced by a single dose of LiCl persisted for 12 months. In contrast, the CG animals continued to consume the plant in all tests performed, indicating the absence of aversion. This study showed that aversive conditioning using LiCl was effective in preventing poisoning by **P. aeneofusca** for a period of at least 12 months.*

Key words: *toxic plants, sudden death, monofluoroacetate, lithium chloride*.

RESUMO

*O objetivo deste trabalho foi descrever os aspectos epidemiológicos, clínicos e patológicos da intoxicação por **Palicourea aeneofusca** em bovinos, no Agreste de Pernambuco, e comprovar se é possível induzir aversão alimentar à intoxicação por **P. aeneofusca** em bovinos criados sob manejo extensivo. Para determinar a ocorrência da intoxicação, foram visitadas 30 propriedades em cinco municípios do Agreste de Pernambuco. Três surtos de intoxicação em bovinos foram acompanhados. Para se induzir aversão alimentar condicionada ao consumo de **P. aeneofusca**, 12 bovinos foram distribuídos aleatoriamente em dois grupos de seis animais cada. Os bovinos foram pesados e receberam, no cocho, folhas verdes de **P. aeneofusca**, na dose de 35mg kg^{-1} de peso corporal, para consumo espontâneo. Os bovinos do GC receberam água (1mL kg^{-1} de peso corporal), via sonda esofágica, após a primeira ingestão da planta, e os demais constituíram o GTA, que foram induzidos à aversão com cloreto de lítio (LiCl - 175mg kg^{-1} de peso corporal), via sonda esofágica. Para os bovinos do GTA, a indução de aversão a **P. aeneofusca**, em que se utilizou dose única de LiCl, persistiu por 12 meses. Por outro lado, os bovinos do grupo GC continuaram ingerindo a planta em todos os testes realizados, indicando a ausência de aversão. Este trabalho comprova que o condicionamento aversivo usando LiCl foi eficaz para prevenir a intoxicações por **P. aeneofusca** por um período de, pelo menos, 12 meses.*

Palavras-chave: *plantas tóxicas, morte súbita, monofluoroacetato, cloreto de lítio.*

INTRODUCTION

Among the ***Rubiaceae*** that contain monofluoroacetate (MFA) (LEE et al., 2012), ***Palicourea aeneofusca*** (Müll. Arg.) Standl., (***Rubiaceae***) is the most important toxic plant for cattle on the coast and northeastern forests of Brazil (TOKARNIA et al., 1983; VASCONCELOS et al.,

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2008). In most cases, the prophylaxis for poisoning by toxic plants in Brazil involves ineffective measures such as avoiding excessive grazing, building fences to isolate infested areas, and elimination of toxic species by manual removal, herbicides, mowing, and plowing. These measures become even more ineffective when a plant is very palatable and causes peracute poisoning, as in the case of poisoning by ***P. aeneofusca*** (RIET-CORREA & MÉNDEZ, 2007).

Recently, conditioned food aversion was used to prevent poisoning by plants in ruminants in Brazil (GORNIAK et al., 2008; OLIVEIRA et al., 2014). Various substances can be used including lithium chloride (LiCl). When administered by a ruminal fistula or feeding tube immediately after consumption of the plant, LiCl induces aversion by producing gastrointestinal discomfort without major side effects (RALPHS et al., 2001). In a previous study in goats, the possibility of inducing conditioned food aversion to ***P. aeneofusca***, which persists for at least 90 days, was demonstrated (OLIVEIRA et al., 2014). However, this methodology has not been tested in cattle raised under extensive management conditions.

The objective of this study was to describe the epidemiological, clinical, and pathological aspects of spontaneous ***P. aeneofusca*** poisoning in cattle in the region of Pernambuco, Brazil and to determine if it is possible to induce conditioned food aversion, to prevent outbreaks of poisoning, through the administration of LiCl immediately after ingestion of nontoxic doses of the plant in cattle under extensive management conditions.

MATERIALS AND METHODS

Epidemiological study

To investigate the occurrence of spontaneous poisoning by ***P. aeneofusca***, an epidemiological study was conducted in the region of Pernambuco in the municipalities of Angelim, Bom Conselho, Canhotinho, Garanhuns, and Jurema. To establish the location for performing the aversion tests, visits were made to six farms bordered by forest areas in each municipality.

The owners or individuals responsible for agricultural activities were interviewed using a questionnaire similar to that used by SILVA et al. (2006), which included questions related to the distribution, period of occurrence, and symptoms caused by ***P. aeneofusca*** poisoning in production animals. Next, the areas of pasture bordered by the Atlantic Forest were inspected for the presence of ***P. aeneofusca***. Rural farms that contained areas with ***P. aeneofusca*** were monitored by the Animal Diagnostic Laboratory of the Universidade Federal Rural of Pernambuco (LDA- UFRPE) for 12 months.

Outbreaks of ***P. aeneofusca*** poisoning

Three outbreaks of spontaneous poisoning by ingestion of ***P. aeneofusca*** occurred in the municipality of Jurema, PE and were monitored by a LDA-UFRPE team during the years of 2012 and 2013. The cattle were all male crosses of the Girolando and Nelore breeds between 12 and 24 months old and were raised in an extensive grazing system. Eight animals that presented more intense clinical signs of poisoning by ***P. aeneofusca*** were examined and necropsied. Organ samples from the abdominal cavity, chest cavity, and central nervous system were collected, fixed in 10% formalin, processed according to routine methods, stained with hematoxylin and eosin (HE), and evaluated histopathologically.

P. aeneofusca leaves were collected in the municipality of Jurema, PE and sent to the Poisonous Plant Laboratory, Agricultural Research Service, United States Department of Agriculture, Logan, UT, USA, for verification of the existence of MFA in their composition according to the methodology described by LEE et al. (2012).

Aversive conditioning

The experiment was conducted in a farm located in the city of Jurema, PE (08°44'38.1"S - 36°08'45.5"W). For the experiment, an area composed of eight hectares of Atlantic Forest that was severely invaded by ***P. aeneofusca*** (more than 20 bushes per hectare) was fenced and divided into two lots, each consisting of four hectares (lots A and B).

The study included 12 male Girolando cattle with an average age of 10 to 18 months that weighed between 165 and 300kg from a region without ***P. aeneofusca***. Animals were transported to the farm for adaptation and preliminary clinical observation 30 days before the start of the experiment. During this period, the animals were kept in a pasture of ***B. decumbens*** with *ad libitum* access to water and mineral salt.

Before the beginning of the experiment, the animals were fasted for 12 hours and then were randomly divided into two groups of six animals each: a control group (CG) and an aversion test group (ATG). Green leaves from ***P. aeneofusca*** were collected in the forest area and then placed in the feeding troughs of cattle in both groups for spontaneous consumption at a dose of 35mg kg^{-1} of body weight. After this procedure, the CG animals received water via a feeding tube at a dose of 1ml kg^{-1} body weight and, after 24 hours, were clinically

evaluated for the presence of poisoning symptoms. If no symptoms were observed, the cattle were released into lot A. The cattle in the ATG group received, via a feeding tube, LiCl diluted in one liter of distilled water at a dose of 175mg kg^{-1} of body weight. After 24 hours, the cattle were evaluated clinically and released into lot B.

The cattle were released to graze in the lots once every 30 days, where they remained for a maximum of 15 consecutive days or until they presented signs of poisoning (isolation from the group, positive venous pulse, reluctance to move, and sternal recumbency). During this period, the cattle were observed daily using binoculars for four consecutive hours as they spontaneously consumed ***P. aeneofusca***. After being removed from lots A and B, the cattle were directed to a pasture of ***Brachiaria decumbens*** with access to water and mineral salt *ad libitum*, where they remained for another 15 consecutive days. This procedure was repeated for 12 months.

RESULTS

Epidemiological study

Incidences of poisoning reported by interviewees occurred on farms with similar characteristics of livestock exploration, comprising areas of pasture of ***Brachiaria decumbens*** and ***Digitaria decumbens***, located in areas bordered by vegetation of the Atlantic Forest. In Jurema, ***P. aeneofusca*** was mentioned five times and said to be responsible for the deaths of 39 cattle. In Canhotinho, no outbreaks were reported, and the plant was not found at the sites visited. In the municipality of Angelim, the plant was mentioned once as being responsible for the deaths of three goats and 17 cattle. In the municipalities of Bom Conselho and Garanhuns, poisoning by ***P. aeneofusca*** was mentioned three times; on these properties, the LDA-UFRPE team found several areas of forest containing ***P. aeneofusca***.

The plant collected in the municipality of Jurema, PE, was identified as ***P. aeneofusca***, and the presence of 0.09% MFA was detected in the leaves of the adult plant.

Outbreaks of ***P. aeneofusca*** poisoning

In the municipality of Jurema, in December of 2012, during the removal of a lot of 42 cattle in a pasture containing Atlantic Forest, 11 cattle showed signs of ***P. aeneofusca*** poisoning, and four died. On the second farm, where 21 cattle were raised, the signs of poisoning were mainly observed in a group of five animals that had invaded a forested area previously isolated by fencing; two cattle died from this group. On this property, sudden deaths of cattle had been reported in previous years. The last observed outbreak occurred in February of 2013. The owner of a herd of 23 cattle had rented a pasture of ***Brachiaria decumbens***, also bordered by forest. Fifteen days after the introduction of cattle, two animals died suddenly. The next day, the remaining cattle were removed from the area, and six cattle presented signs of poisoning along the way but recovered within 24 hours after the onset of clinical signs. In all the farms inspected, examples of ***P. aeneofusca*** were encountered (Figure 1A and 1B).

The clinical signs of poisoning by ***P. aeneofusca*** in the examined cattle were uniform and mainly consisted of lethargy, prolonged sternal recumbency, lateral decubitus, reluctance to move or get up, fatigue, tachypnea, tachycardia, and positive venous pulse. Animals that were forced to move showed instability, muscle tremors and a single fall followed by vocalizations, paddling movements, and death in approximately 5 to 10 minutes.

In macroscopic examinations of eight intoxicated cattle, no significant lesions were observed. Microscopically, cardiac abnormalities were observed in five cattle. Diffuse areas of hemorrhage, edema of the cells of Purkinje fibers, and coagulative necrosis of myocardial fibers were observed. Abnormalities in these tissues consisted of fibers in which the nucleus was undergoing karyolysis or pyknosis, increased cytoplasmic eosinophilia, and loss of striations. In the fibers, discrete intranuclear vacuoles were observed with marginalization of chromatin. In the interstitial spaces, discrete edema, fibrin deposits, and mononuclear cell infiltration were observed.

In one case, a histopathological examination of the kidneys did not show abnormalities. In the other cases, the histopathological examination showed congestion, cytoplasmic hydropic changes or vacuolar degeneration (HVD), and necrosis of varying degrees of severity in epithelial cells of the renal convoluted tubules. In some tubules, necrosis was not observed; only HVD or intermediate stages that included degeneration and incipient necrosis were observed.

Aversive conditioning

During the initial observation period in the corral, none of the CG cattle showed signs of poisoning after consuming ***P. aeneofusca*** leaves at a dose of 35mg/kg body weight. On the fifth day of the experiment, two cattle from this group showed signs of poisoning and died. On that occasion, the other

Figure 1 - A) P. aeneofusca in an altitudinal wetland forest in Jurema-PE, northeastern Brazil. B) P. aeneofusca showing signs of consumption by cattle.

cattle were observed consuming the plant and were removed from the forest. During the next 12 months of observation of the control pasture, every animal from the CG, when allowed to graze in forested areas that contained ***P. aeneofusca***, began to ingest the plant and was removed from the pasture.

All ATG cattle spontaneously consumed ***P. aeneofusca*** leaves in their troughs at a dose of 35mg kg^{-1} body weight. During the initial aversive treatment performed in the corral, no cattle in this group showed clinical signs after administration of the plant or after administration of LiCl. After being released into the lot invaded by ***P. aeneofusca***, none of the cattle ingested the plant. As they approached the bushes, the cattle smelled the plant without consuming it.

For the ATG cattle, only one dose of LiCl was necessary, and the food aversion to ***P. aeneofusca*** persisted throughout the entire test period in the field, totaling 12 months. In contrast, the CG cattle ingested the plant in all tests, indicating the absence of conditioning. For all animals, the identification number, body weight, and treatments performed are presented in table 1.

DISCUSSION

The diagnosis of poisoning by ***P. aeneofusca*** in cattle was based on epidemiological findings (the occurrence of sudden death in animals with symptoms of consumption that had grazed in areas that contained large amounts of ***P. aeneofusca*** and the absence of other plants on the property able to cause the same clinical symptoms), clinical signs, and histopathological abnormalities.

No abnormalities were observed macroscopically. However, it is known that cattle consuming repeated doses of plants containing MFA may exhibit rounded greyish-white or reddish-white lesions in the papillary muscles (TOKARNIA et al., 1985; BANDINELLI et al., 2014). One frequently observed histological lesion was the necrosis of myocardial fibers. This lesion has previously been described in cattle poisoned by other plants containing MFA (TOKARNIA et al., 1985; PAVARINI et al., 2011). Necrotic lesions can be observed that are focally extensive, accentuated in the papillary

Table 1 - Animal identification, weight, amount of green ***P. aeneofusca*** leaves, water, and LiCl administered to the control and aversion test groups during the initial treatment.

Group	Identification	Body weight (kg)	Green leaves of P.a (kg)	Water administered (ml)	LiCl administered (ml)
Control	Bovine 1*	220	0.07	220	-
	Bovine 2	180	0.06	180	-
	Bovine 3	300	0,1	300	-
	Bovine 4	174	0.06	174	-
	Bovine 5*	200	0.07	200	-
	Bovine 6	165	0.05	165	-
Aversion test group	Bovine 7	188	0.06	1000	188
	Bovine 8	174	0.06	1000	174
	Bovine 9	185	0.06	1000	185
	Bovine 10	185	0.06	1000	185
	Bovine 11	200	0.07	1000	200
	Bovine 12	200	0.07	1000	200

*Animals that died; P.a = ***Palicourea aeneofusca***; LiCl = Lithium Chloride.

muscles, and bordered by halos with inflammatory infiltrate composed of macrophages, lymphocytes, and degenerate neutrophils, as well as fibrosis, similar to that observed after a stroke (BANDINELLI et al., 2014). These lesions should be taken into consideration because, when present, they are of diagnostic value (TOKARNIA et al., 1991), similar to the HVD of epithelial cells of renal tubules (SILVA et al., 2006).

In the municipalities of Jurema, Angelim, and Bom Conselho, the occurrence of ***P. aeneofusca*** associated with cases of poisoning in cattle had not yet been reported. Properties of these cities have ecoregions of altitudinal wetland forests in common. These regions are located in the semi-arid northeast that, due to the high altitude, have a humid and sub-humid tropical climate. These aspects create the conditions necessary for the development of vegetation with both Atlantic Forest and Caatinga characteristics (PÔRTO et al., 2004).

Under natural conditions, cattle are poisoned due to the high palatability of ***P. aeneofusca*** and not due to necessity or hunger (TOKARNIA et al., 1983). Cases of poisoning can occur at any time of year whenever cattle enter into forested areas (TOKARNIA et al., 1983; RIET-CORREA et al., 2011). In the region examined in this study, cases of poisoning were concentrated mainly in the dry season, which extends from September to February. Despite the risks, forested areas are often the only option for cattle grazing.

Regarding the experiment with cattle, we found that all of the CG animals showed a preference for ***P. aeneofusca*** leaves; they readily consumed the plant, and the only reason that they did not all die was because they were removed from the forested area as soon as consumption of the plant was observed. The results obtained in this study from ATG cattle showed that under extensive management conditions, the technique of conditioned food aversion, using LiCl according to the methodology proposed in this study, can be effectively implemented to prevent poisoning by ***P. aeneofusca***.

In previous studies in which LiCl was used to prevent poisoning in cattle by ***Delphinium barbeyi***, it has been shown that the aversive effect lasted for at least three years (RALPHS & PROVENZA, 1999). In recent experiments on goats, conditioned food aversion was used as a method to control poisoning by ***Ipomoea carnea*** subsp. ***fistulosa*** (PIMENTEL et al., 2012). It was shown that goats that were recently adapted to ingestion of this plant and subjected to aversive treatment with LiCl did not ingest the plant for at least 24 months. In Brazil, in experiments with goats and sheep, LiCl was also used to induce aversion to the consumption of ***Amorimia rigida*** (PACÍFICO DA SILVA & SOTO-BLANCO, 2010) and ***Leucaena leucocephala*** (GORNIAK et al., 2008).

The mechanism responsible for the development of aversion has not been established, although it has been suggested that aversion may be the result of a combination of sensory information (distinct aroma of the food) and feedback signals (nutritional properties and toxic effects on chemo, osmo, and mechano-receptors) unique to each food. In this sense, LiCl acts by affecting the emetic center

of the brain and causing nausea in cattle (RALPHS & PROVENZA, 1999; RALPHS et al., 2001).

CONCLUSION

It was demonstrated that ***P. aeneofusca*** is responsible for mortality of cattle in the middle region of Pernambuco and that aversive conditioning was effective in eliminating the tendency of cattle to consume ***P. aeneofusca*** for a period of at least 12 months.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experiment was approved by the committee of ethics on the use of animals of Universidade Federal Rural de Pernambuco (UFRPE) (protocol n. 057/2013).

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