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EFECTO GENOTÓXICO DE ANTIPARASITARIOS SELECCIONADOS EN *Caiman latirostris*

GENOTOXIC EFFECTS OF SELECTED ANTIPARASITIC DRUGS IN *Caiman latirostris*

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RESUMEN

El yacaré overo (*Caiman latirostris*) es un reptil autóctono del centro-norte de Argentina y otros países de Sudamérica. Sus poblaciones se ven afectadas por la pérdida y modificación del hábitat debido a la expansión agropecuaria. La intensificación ganadera introdujo un mayor uso de drogas de uso veterinario, especialmente antiparasitarios (APs). Estos compuestos pueden contaminar suelos y cuerpos de agua cercanos, representando un riesgo para los caimanes. El objetivo de este estudio fue evaluar los efectos genotóxicos potenciales de las formulaciones comerciales de albendazol, fenbendazol e ivermectina en juveniles de *C. latirostris*. Se realizó una exposición subcrónica durante dos meses a 48 individuos que fueron distribuidos equitativamente en 4 tratamientos (12 individuos en cada grupo): Control Negativo (CN) tratado con agua potable; un grupo expuesto a albendazol (Zoovet 3,8% -20 ng/mL), otro a fenbendazol (Zoovet 5% - 20 ng/mL) y un grupo expuesto a ivermectina (Zoovet 1% - 10 ng/mL). Al finalizar el ensayo se tomaron muestras de sangre a todos los individuos para evaluar genotoxicidad a través de la prueba de micronúcleo y otras anormalidades nucleares, y del ensayo cometa. Los resultados indicaron un daño en el ADN significativamente mayor en los individuos expuestos a ivermectina en comparación con el CN. No se encontraron diferencias significativas en el resto de los marcadores de genotoxicidad en los grupos expuestos. Este es el primer estudio en evaluar los posibles efectos genotóxicos de los AP en esta especie de reptil. Los resultados obtenidos nos alertan sobre el potencial impacto de la ivermectina en nuestros ecosistemas.

Palabras clave: cocodrilianos, contaminación ambiental, drogas de uso veterinario, avermectinas, benzimidazoles

ABSTRACT

The broad-snouted caiman (*Caiman latirostris*) is a reptile native to north-central Argentina and other countries in South America. Many populations are affected by habitat loss and modification associated with agricultural expansion. Livestock intensification has led to increased use of veterinary products, particularly antiparasitic drugs (APDs). These compounds may contaminate nearby soil and water bodies, posing a potential risk to caimans. The aim of this study was to evaluate the potential genotoxic effects of commercial formulations of albendazole, fenbendazole, and ivermectin in juvenile *C. latirostris*. A subchronic exposure was conducted over two months using 48 individuals, which were distributed into four treatments (12 individuals per group): a Negative Control (NC) treated with drinking water; a group exposed to albendazole (Zoovet 3.8%– 20 ng/mL), another exposed to fenbendazole (Zoovet 5% – 20 ng/mL) and a group exposed to ivermectin (Zoovet 1% –10 ng/mL). At the end of the experiment, blood samples were collected from all individuals to assess genotoxicity through the micronucleus and nuclear abnormalities tests, as well as the comet assay (CA). The CA revealed significantly higher DNA damage in caimans exposed to ivermectin compared to NC. No significant differences were found for the other genotoxicity biomarkers in any of the exposed groups. This is the first study to assess the potential genotoxic effects of APDs in this reptile species. These findings highlight the potential environmental impact of ivermectin on ecosystems.

Key words: crocodiles, environmental contamination, veterinary pharmaceuticals, benzimidazoles, avermectins

INTRODUCTION

In Argentina, the expansion of the agricultural frontier resulted in the loss and degradation of natural habitats and led to a significant shift in land use (Gaona *et al.,* 2019). At the same time, cattle production was transformed and intensified to increase profitability. Consequently, traditional pastoral systems were replaced by intensive feedlot systems or semi-intensive systems supplemented with pasture (Pordomingo, 2013). This practice, along with a greater concentration of livestock in areas with high environmental fragility, favors disease transmission, thus generating new sanitary requirements such as increasing the use of antiparasitic drugs (APDs) (Miranda, 2014). APDs are broad-spectrum veterinary drugs that are routinely used in livestock farming to control parasites (both internal and external; Boxall, 2010). Furthermore, intensive livestock operations are characterized by poor infrastructure and low investment, with compacted earthen floors that allow contaminants to seep into groundwater (Pordomingo, 2013; Salahuddin and Shaharyar, 2017). Consequently, APDs can be released into the environment and impact organisms inhabiting areas affected by livestock activities (Mesa *et al.,* 2020). Among the most used PADs are benzimidazoles (e.g. albendazole and fenbendazole) and avermectins (e.g. ivermectin). Specifically, albendazole and fenbendazole are broad-spectrum systemic anthelmintics and are therefore commonly used against cestodes, nematodes, and acanthocephalans (Dar *et al.,* 2018). On the other hand, ivermectin is a semisynthetic derivative of avermectin, an macrocyclic lactone, used for the intestinal control of nematodes and ticks (El-Saber Batiha *et al.,* 2020). Although APDs have been used for many years, their environmental impact is largely unknown (Mesa *et al.,* 2020). The original compounds and/or their metabolites are typically excreted in urine and feces and released into the environment in pastures or stored in manure in the soil, eventually reaching nearby waterways through runoff or leaching from surrounding fields. Globally, benzimidazoles and avermectins have frequently been found in the ng/mL range when surface waters were monitored (Iglesias *et al.,* 2012; Ugonna Odo *et al.,* 2020; Belew *et al.,* 2021).

The impact of exposure to APDs drugs on wildlife may be reflected in changes in cellular metabolism. To date, there are few reports on the effects of benzimidazoles and avermectins on native species inhabiting natural areas affected by livestock activities (Ogueji *et al.,* 2020). Among benzimidazoles, Park *et al.* (2009) evaluated the effects of prolonged fenbendazole exposure on molecular and biochemical responses in *Chironomus riparius*. The expression of heat shock protein (*hsp70, hsp40, hsp90*), and cytochrome P450 genes increased significantly at all tested concentrations, whereas glutathione S-transferase activity increased only in organisms exposed to the highest fenbendazole concentration (30 µg/L). Consistent with these findings, fenbendazole has been shown to cause ecotoxic effects in several aquatic organisms, including *Vibrio fischeri*, *Scenedesmus vacuolatus*, *Lemna minor*, and *Daphnia magna*, with the latter being the most sensitive species (48-h EC_{50} = 19 µg/L), underscoring the need to assess its potential chronic effects on aquatic ecosystems (Wagil *et al.,* 2015a). Similarly, Carlsson *et al.* (2018) evaluated whether exposure to APDs, including fenbendazole, affected swimming activity in 6-day-old zebrafish larvae, finding a reduction in swimming activity. Adverse effects have also been reported for avermectins. Mesa *et al.* (2017) exposed aquatic invertebrates (*Ceriodaphnia dubia, Hyalella, and Pomacea*) to different concentrations of ivermectin (22, 50, 458, and 1150 µg/kg) and noted its lethal toxicity in exposed organisms and persistence in sediments over an extended period of time. At the molecular level, Villada-Bedoya *et al.* (2021) reported that ivermectin increased the expression levels of *hsp70* as well as antioxidant capacity in the *Euoniticellus intermedius*. In juvenile *Clarias gariepinus* fish, exposure to ivermectin (0.007, 0.013, and 0.033 mg/L) induced oxidative damage to lipids and altered the activity of antioxidant enzymes (SOD and CAT) in the brain, liver, and gills (Ugonna Odo *et al.,* 2020). On the other hand, Nie *et al.* (2022) evaluated the effects of environmentally relevant concentrations of abamectin (0.02 mg/L and 2 mg/L) on *Eremias argus* lizards following a 30-day exposure and reported increased oxidative stress, alteration in locomotion, and changes in behavior related to predation.

The broad-snouted caiman (Crocodylia, Alligatoridae, *Caiman latirostris*) is one of two native crocodilian species in Argentina, with a range that covers a large region of the north-central part of the country (Larriera *et al.,* 2008). As an ectothermic organism, the caiman has a defined seasonal cycle that depends on two main climatic factors, temperature and precipitation; furthermore, its lifestyle is necessarily associated with water (Poletta *et al.,* 2017). Caiman populations are potentially exposed to complex mixtures of different products used in agricultural activities, including pesticide and APDs formulations, which represent a high risk to the development of embryos and neonates (Poletta *et al.,* 2017). In the long term, these effects could result in a decline in *C. latirostris* populations, potentially altering the food web within its habitat by modifying predator–prey relationships, changing the abundance of certain prey species, and indirectly affecting other trophic levels. Additionally, due to its longevity and strong fidelity to its habitat, *C. latirostris* is considered an excellent sentinel organism for studying and monitoring the consequences of exposure to xenobiotics released into the environment by human activity (Poletta *et al.,*

2009). In previous studies, we have demonstrated that pesticides induce oxidative stress through alterations in the activity of antioxidant enzymes (SOD and CAT), lipid peroxidation, oxidation of nitrogenous bases, and DNA damage in neonates and juveniles of *C. latirostris*, even at concentrations recommended for crop application (Poletta *et al.,* 2017; López González *et al.,* 2021, 2022; Odetti *et al.,* 2020, 2023). Currently, natural populations of caimans have been found in water bodies near facilities where intensive livestock farming takes place; as a result, they may be exposed to residues of the AP drugs used in these systems. However, the potential effects of these drugs on this species, or on any other crocodilian species worldwide, have not yet been studied. Considering this lack of knowledge and the potential toxicity of widely used veterinary products in livestock production, the aim of this study was to evaluate alterations in the genetic material in erythrocytes of *C. latirostris* following sub-chronic exposure to the APDs drugs albendazole, fenbendazole, and ivermectin.



Figure 1. *C. latirostris* yearling (6 months age, 30 cm total length). Picture by Pablo Siroski.

MATERIALS AND METHODS

Animals

The procedures were approved by the Advisory Committee on Research Ethics and Safety (FBCB-UNL), Act No. 1/22 dated March 10, 2022. The animals used in this study were obtained from eggs collected at the Managed Natural Reserve "El Fisco" (30°11′26″S, 61°0′27″W; San Cristóbal Department, Santa Fe, Argentina), a protected natural area (Law 12,930, 2008) covering approximately 1,800 hectares. This area was selected because it is part of the natural distribution of the species and, to date, no agricultural, livestock, or urban activities that could be a source of contamination are carried out there. Moreover, this area has been previously used as a reference (control) site in several studies (Poletta *et al.,* 2008, 2009; Odetti *et al.,* 2020; López González *et al.,* 2021, 2022). The eggs were transported following standard procedures to the facilities of the Center for Rescue, Research and Interpretation of the Fauna "La Esmeralda" (CRIIF, Santa Fe, Argentina), where they were incubated under controlled conditions. After hatching, individuals from each clutch were uniquely identified using a combination of cuts in single and double caudal scutes. The animals were then reared under controlled conditions until the beginning of the experiment (Figure 1) (Poletta *et al.,* 2017; Siroski *et al.,* 2024).

Experimental design and treatments

To evaluate the potential effects of albendazole, fenbendazole, and ivermectin on 6 months old caimans were used, originating from eggs collected at the beginning of the reproductive season in the previously described natural area. An exposure assay by voluntary immersion was conducted following the methodology described for the species by López González *et al.* (2021). Animals from each experimental group were maintained in plastic containers measuring 55 cm in length, 35 cm in width, and 27 cm in height (0.20 m^2 base area). The containers were placed tilted to provide a 60% water and 40% dry surface ratio, with a fixed volume of 4 L (dechlorinated drinking water) and a maximum depth of approximately 15 cm, located inside a heated room (30 ± 2 °C). The temperature of each container was recorded using a HOBO Temp data logger (Onset Computer Corporation, Pocasset, MA, USA) to prevent it from fluctuating outside the previously specified range.

Exposure was performed by voluntary immersion, through the incorporation of the compounds into the water in the containers. The experiment was conducted for 60 days, classified as subchronic (Eaton and Gilbert, 2008), with water and solution changes every 48 hours, coinciding with the cleaning schedule. The specimens were randomly distributed into four experimental groups of 12 animals (each with 2 replicates of 6 individuals, total N=48), leaving them to acclimatize for one week. The treatments were as follows: 1) a negative control (with dechlorinated tap water), without exposure to the drugs, 2) a group exposed to the APD albendazole (Zoovet 3.8% – 20 ng/mL), 3) one exposed to fenbendazole (Zoovet 5% – 20 ng/mL), and 4) one to ivermectin (Zoovet 1% - 10 ng/mL). These concentrations were selected based on a range similar to those reported by various studies under controlled conditions and for environmental residues (Carlsson *et al.,* 2013; Belew *et al.,* 2021; Lozano *et al.,* 2021; Muniz *et al.,* 2023). During the study, the animals were fed three

times a week with a mixture consisting of 50% ground chicken heads and 50% dry reptile pellets, provided *ad libitum* (Odetti *et al.,* 2023). Container cleaning was alternated with feeding days, and APDs solutions were renewed after each water replacement.

Antiparasitics determination

The nominal concentration of APDs in the formulations was determined by high-performance liquid chromatography (HPLC) at Leon Pharma Laboratories. HPLC conditions for Albendazol/Fenbendazol were: temperature: 35°C, injection volume 100 µl, flow volume 0.7 ml/min, wavelength 270 nm, time of running 20 min.; and for Ivermectin were: temperature 30°C, injection volume 100 µl, flow volume 1.4 ml/min, wavelength 254 nm, time of running 20 min. The percentage of recovery was 96.6% for ivermectin, 95.5% for albendazole and 98.8% for fenbendazole.

Biomarkers of genotoxicity

Sampling

At the end of the exposure period, 1 mL of peripheral blood was taken from the spinal vein of each specimen with a heparinized syringe and TERUMO® 25 G x 5/8" (0.5 mm) needle (Myburgh *et al.,* 2014) and immediately used for genotoxicity testing.

Comet assay

The alkaline comet assay (CA) was performed on *C. latirostris* erythrocytes according to the protocol adapted for the species by Poletta *et al.* (2008). All samples were coded for blind analysis, slides were stained with acridine orange and images were analyzed under a fluorescence microscope (Olympus CX 40 equipped with a URFLT 50 excitation filter). Images of 100 randomly selected nucleoids were visually classified into four arbitrary damage categories (1 = slight damage; 2 = moderate damage; 3 = high damage and 4 = extremely high damage) according to tail size and intensity. The DNA damage index (ID = n1 + 2 n2 + 3 n3 + 4 n4) was calculated for each animal, where n is the number of nucleoids in each category (Figure 2) (Poletta *et al.*, 2008).

Micronucleus assay and other nuclear abnormalities

The micronucleus (MN) and other nuclear abnormalities (NAs) tests were performed on *C. latirostris* erythrocytes according to the techniques described by Poletta *et al.* (2008) and Lopez Gonzalez *et al.* (2017). Immediately after sampling, a small amount of blood from each animal was smeared over two slides, fixed with ethanol for 10 min and stained with Giemsa (10%) for 15 min. A total of 1,000 erythrocytes per animal were analyzed under the optical microscope mentioned before at 1000x magnification and the frequencies of MN/NAs were determined (FMN/FNAs: number of cells with MN or other NAs/1000 erythrocytes counted). The NAs were classified in five categories: notched nuclei (NN, appreciable depth into a nucleus that does not contain nuclear material), nuclear buds or "budding" (nuclear evaginations), binucleated cells (BiN, cells undergoing incomplete cell division, with two completely separate nuclei in the same erythrocyte cytoplasm), eccentric nuclei (EN, cells with the nucleus in an abnormal peripheral position), and the sum of total NAs observed (TNAs).

Data analysis

The SPSS 15.0 software for Windows (SPSS, 2019) was used to conduct statistical analysis. All variables were compared for replicates using One-way ANOVA. Since no differences were found among the replicates, the experimental groups were considered as the grouping variable for the analysis. For all the parameters analyzed, mean values ± standard error (SE) were calculated from the data. Variables were tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). Data were analyzed using the Kruskal–Wallis test followed by Dunn–Bonferroni post hoc comparisons when significant differences were detected ($p < 0.05$) for MN, NAs and CA.

RESULTS

The results obtained in the CA showed a significantly higher level of DNA damage in the group treated with ivermectin compared to the control ($p = 0.0291$). In contrast, no significant differences were observed for the benzimidazoles treatments relative to the control (Figure 4).

Regarding the results obtained from the MN and NAs tests, no statistically significant differences were observed between the exposed groups and the control ($p > 0.05$, Table 1).

When results were expressed as percentages relative to negative control, considered as 100%, a higher percentage of DNA damage was observed in all treatments with the CA. Ivermectin showed the highest value, reaching 127.28%, followed by fenbendazole at 116.33% and albendazole at 113.27%. A similar pattern was observed for MN, with all treatments showing values above the NC. The highest value was again recorded in the ivermectin, reaching 282.25%, followed by albendazole at 241.93% and fenbendazole at 220.96%.

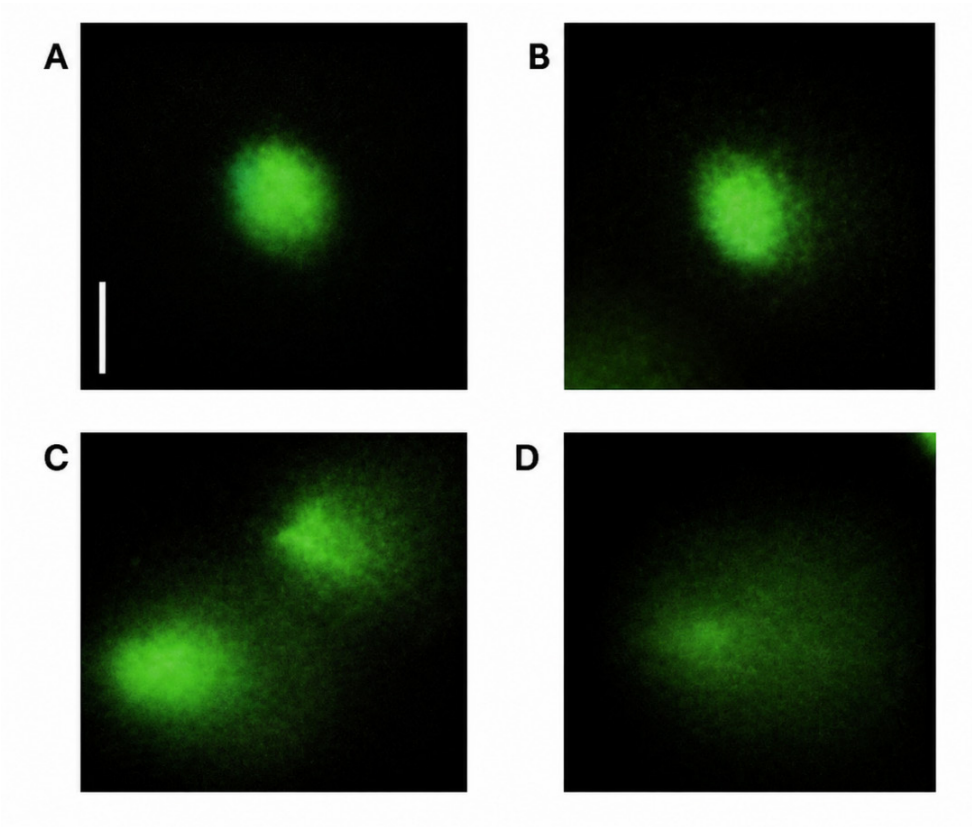


Figure 2. Comet assay visual analysis. Different classes of damage observed in peripheral blood erythrocytes of *Caiman latirostris*. A: class 1; B: class 2; C: class 3; D: class 4. Scale bar = 10 µm.

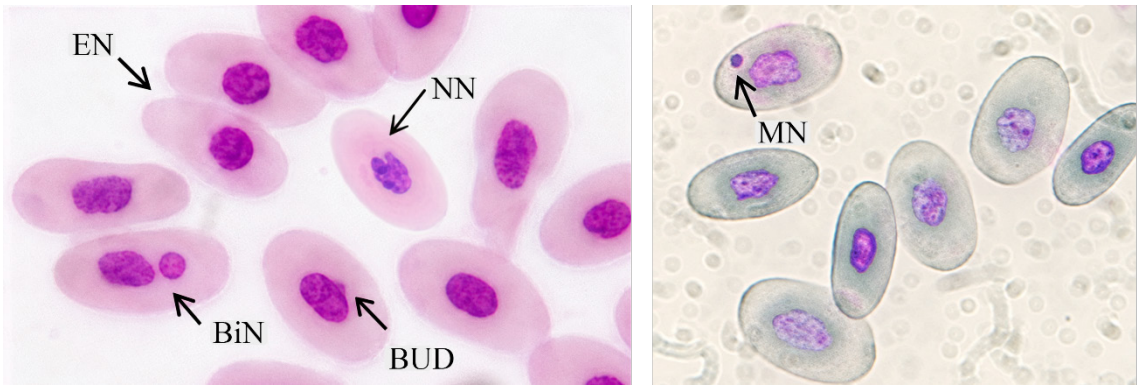


Figure 3. Images of some of the nuclear abnormalities analyzed in peripheral blood erythrocytes of *C. latirostris*. MN: Micronucleus; NN: notched nuclei; Buds: nuclear bud; BiN: binucleated erythrocyte; EN: eccentric nucleus. 1000×.

DISCUSSION

In recent decades, environmental contamination by APDs has emerged as a growing global concern, mainly due to their intensive use in livestock production systems. The presence of these compounds has been reported in several environmental compartments, including surface waters, groundwater, soils, sediments, and biota (Mesa *et al.*, 2020; Lozano *et al.*, 2021; Muniz *et al.*, 2023). This scenario is closely associated with unsustainable anthropogenic practices and may have particularly severe consequences for wildlife (Hooman, 2020). In this context, and considering their widespread application, APDs, together with other contaminants derived from agricultural and livestock activities, are increasingly recognized as relevant stressors for reptile populations exposed to contaminated environments (Sasaki *et al.*, 2015). Among ADPs, albendazole,

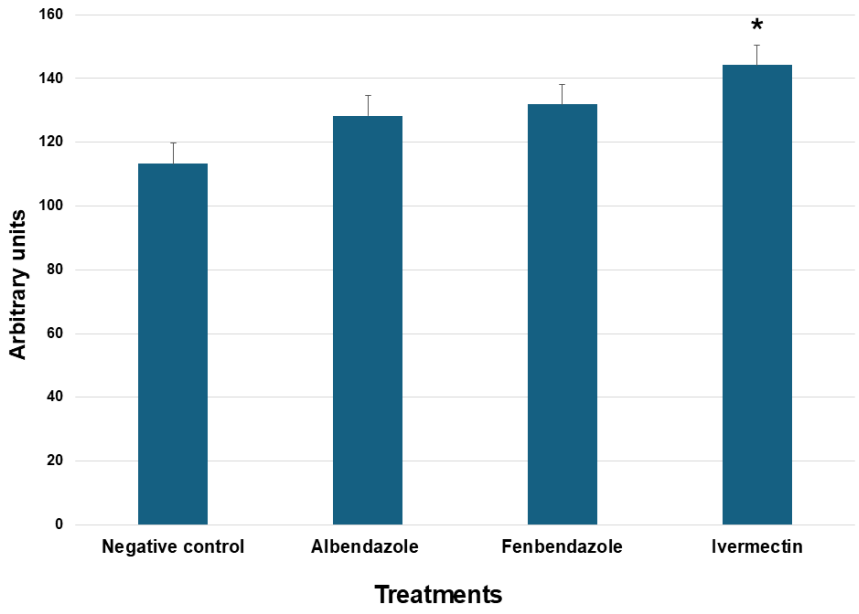


Figure 4. DNA damage index detected by the Comet assay in *Caiman latirostris* hatchlings from different treatments.

fenbendazole and ivermectin, have been reported in different environmental matrices, but the available information on their ecotoxicological effects remains comparatively limited (Wagil *et al.*, 2015b; Muniz *et al.*, 2023).

In north-central Argentina caiman populations face one of the main challenges that has affected many wild species for several years: habitat loss as a consequence of increasing area dedicated to agricultural production. In this sense, land use in the floodplain of the middle Paraná River has changed significantly in recent decades, as the intensification of agriculture has forced the relocation of livestock to marginal sites, increasing livestock density (Quintana *et al.*, 2014). The consequent widespread use of these drugs has raised concerns about the ecotoxicological risks they may generate in environments increasingly impacted by livestock farming activities (Mesa *et al.*, 2020).

To date, the effects of the APDs have not yet been reported in *C. latirostris* or in any other crocodilian species. In this work, yearling caimans were used, because during their first year of life they spend most of their time in the water and typically inhabit shallow water bodies, many of which may be contaminated due to their proximity to crops, feedlot systems, or both, thus acting as sites of discharge and accumulation of substances derived from these activities. Moreover, at this stage of the life cycle, general defense systems are still under development (Poletta *et al.*, 2009; Weir *et al.*, 2010).

Genetic damage caused by different toxic compounds can disrupt normal cellular processes and interact directly or indirectly with DNA, leading to genetic instability (Poletta *et al.*, 2017). The CA is a sensitive, rapid, and simple technique for detecting early stages of DNA damage at the level of an individual cell (Azqueta *et al.*, 2014). It is used to analyze single- and double-strand DNA breaks, alkali-labile sites, DNA–DNA and DNA–protein crosslinks, as well as single-strand breaks associated with DNA repair mechanisms. On the other hand, the increase in the frequency of nuclear abnormalities (MN, Buds, NN, EN and BiN), is considered an early warning marker of clastogenesis and aneugenesis. These structures observed in erythrocytes are thought to represent either fragments of genetic material not incorporated during cell division or damaged DNA that the cell attempts to eliminate through internal repair mechanisms, but which, due to failures or interruptions in exocytosis, remain at the periphery of the nuclear membrane, giving rise to different formations (Harabawy and Mosleh, 2014; Melo *et al.*, 2014). Considering that these biomarkers generally reflect accumulated or persistent genetic damage, the absence of significant differences may be explained by the activation of repair mechanisms that could have reversed the initial damage (not evaluated in this study), an effective mechanism to remove damaged cells from circulation or by the existence of other mechanisms of damage different from those leading to clastogenesis or aneugenesis (Botelho *et al.*, 2015). Recent studies in fish, amphibians, and insects have shown that ivermectin, can induce genetic damage, oxidative stress, alterations in antioxidant enzymes, and changes in the expression of genes encoding heat shock proteins. These studies have been conducted under both controlled conditions (using sublethal concentrations) and field conditions

Table 1. Frequency of micronuclei and other nuclear abnormalities (mean ± SE) observed in erythrocytes of *Caiman latirostris* exposed to ivermectin, albendazole, fenbendazole and negative control.

Experimental group	FMN	Buds	NN	BiN	EN	TNAs
Negative control	0.62 ± 0.26	125.62 ± 17.74	35.5 ± 7.49	0.25 ± 0.25	98.50 ± 14.71	260.50 ± 34.03
Albendazole	1.5 ± 0.65	114.25 ± 12.61	31.87 ± 7.92	0.37 ± 0.18	97.50 ± 16.58	245.50 ± 29.98
Fenbendazole	1.37 ± 0.37	114.25 ± 13.98	32.75 ± 6.27	0.25 ± 0.25	68.62 ± 7.86	217.25 ± 22.49
Ivermectin	1.75 ± 0.36	122.50 ± 12.08	29.75 ± 4.42	0.62 ± 0.32	90.62 ± 14.42	245.25 ± 24.69

MN: Micronucleus; Buds: nuclear bud; NN: notched nuclei; BiN: binucleated erythrocyte; EN: eccentric nucleus; TNAs: total nuclear abnormalities.

(Ogueji *et al.,* 2020; Villada-Bedoya *et al.,* 2021; Lorente *et al.,* 2023; Lozano *et al.,* 2021; Pelusso *et al.,* 2023, 2025). Especially, Peluso *et al.* (2024) evaluated the lethal and sublethal toxicity of glyphosate and ivermectin, both individually and in combination, using environmentally relevant concentrations on *Rhinella arenarum* larvae. The commercial ivermectin formulation (0.00125 mg/L) was more toxic than glyphosate (0.7 mg/L), and combined exposure produced synergistic effects, as well as alterations in oxidative stress and neurotoxicity biomarkers. This study demonstrated that the simultaneous presence of both compounds in agricultural water bodies may generate negative effects on aquatic organisms. On the other hand, Lozano *et al.* (2024) evaluated the sublethal effects of environmentally relevant concentrations of ivermectin in sediments (2 and 20 µg/kg) on juveniles of *Prochilodus lineatus*. Exposure for 14 days produced alterations in oxidative stress and neurotoxicity biomarkers in brain, gill, and liver tissues, including decreased reduced glutathione levels and acetylcholinesterase activity.

In Argentina, only a limited number of studies have investigated the presence of APDs in aquatic ecosystems and sediments. Mesa *et al.* (2020) conducted a field study assessing the presence and concentration of ivermectin in two shallow wetlands of the Paraná River subjected to different livestock uses. The maximum concentration of this compound reported in sediments (17.1 µg/kg wet weight) was seven times higher than the values estimated by Liebig *et al.* (2010) for aquatic systems environmentally exposed to APDs residues (2.4 µg/kg wet weight). Peluso *et al.* (2023) studied the lower Paraná River basin, an important agroproductive region where crop cultivation and farming predominate. In this context, they analyzed the impacts of these activities on a stream in Buenos Aires province by evaluating physicochemical parameters (metals, pesticides, and emerging contaminants) and ecotoxicological responses using larvae of *R. arenarum*, a native amphibian species. Among their findings, ivermectin was detected in water samples, although below the quantification limit, while a concentration of 8.03 mg/kg was recorded in sediments. Considering that caimans are species that move through the water column and can even rest on sediments, our findings on the genetic alterations produced by low concentrations of ivermectin in water, are of concern. Similarly, Teglia *et al.* (2025) evaluated the presence of emerging contaminants associated with agricultural and livestock activities along the Salado River (Santa Fe province, Argentina). In this study, compounds such as albendazole, fenbendazole, ciprofloxacin, enrofloxacin, and thiamethoxam were detected. In particular, albendazole and fenbendazole were found in river samples and wastewater at concentrations ranging from 128 to 1,610 ng/L. Particularly for river samples, fenbendazole was detected at a concentration of 967 ng/L, a level much higher than that tested in our study, so it is necessary to continue studying the possible effects of these compounds in wild species at environmentally relevant concentrations.

In this study, a two-month subchronic exposure was conducted using 48 six-month-old individuals. This represents a strength of the experimental design by allowing the assessment of prolonged exposure effects during an early developmental stage. Three APDs were evaluated: albendazole, fenbendazole, and ivermectin. The concentrations used were selected within a range according to environmental residues and also reported in previous studies under controlled conditions (Carlsson *et al.*, 2013; Belew *et al.*, 2021; Lozano *et al.*, 2021; Muniz *et al.*, 2023), thereby providing environmental relevance to the study. Nevertheless, the use of a unique concentration per compound does not allow dose-response relationships to be established so it constitutes a limitation of the study. Another limitation is the lack of information on the effects of mixtures of APDs and pesticides, which remains largely unexplored. Even so, the approach used provides a valuable first experimental approximation for assessing the potential subchronic effects of environmentally relevant APDs in caimans.

Considering the lack of information, the use of *in vivo* assays under controlled exposure conditions allows us to contribute further insights to the integrated assessment of the effects and mechanisms of APDs

toxicity and to draw conclusions about the deleterious effects observed at the organismal level. Besides, they help to identify the most suitable biomarkers for field studies, particularly in terms of their specificity and sensitivity (Carballo and Mudry, 2006). Based on these studies, relevant information can be obtained to inform decisions on how to mitigate the effects on natural populations of *C. latirostris* naturally exposed to various pollutants. Moreover, these evaluations should consider different life stages of the species that present a real risk of exposure under natural conditions. It is also essential to incorporate a battery of biomarkers representing different levels of biological organization in order to provide a comprehensive assessment of the health status of caiman populations and to more accurately evaluate the toxicological effects of APDs (Carballo and Mudry, 2006; Odetti *et al.*, 2023).

In this study, significant genotoxic effects were observed only in the ivermectin treatment, whereas albendazole and fenbendazole did not produce detectable effects under the tested conditions. In this sense, having demonstrated that ivermectin induced DNA damage at concentrations potentially found in the environment, it is considered of great importance to continue investigating the effects of these compounds at wider concentrations and through other biomarkers, to better understand the magnitude of the effects of these APDs on natural populations of *C. latirostris*. This information is essential for informed decision-making regarding the management and conservation not only of this species, but also of the whole ecosystem.

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AUTHOR CONTRIBUTION STATEMENT

Odetti LM: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. López González EC: Visualization, Methodology. Lorenzi AC: Visualization, Methodology, Investigation. Simoniello MF: Supervision, Resources, Conceptualization. Poletta GL: Writing – review & editing, Supervision, Resources, Investigation, Conceptualization.