COPROPARASITOLOGICAL DIAGNOSIS OF ALLIGATORS *(Caiman latirostris* DAUDIN, 1802) COMMERCIALLY RAISED IN RIO DE JANEIRO

DIAGNÓSTICO COPROPARASITOLÓGICO DE JACARÉS (Caiman latirostris DAUDIN, 1802) CRIADOS COMERCIALMENTE NO RIO DE JANEIRO

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SUMMARY

The aim of this study was to determine the genera of parasites found in fecal samples of commercially raised (farming system) alligators (*Caiman latirostris* Daudin, 1802) in Rio de Janeiro state, in the period 2008 to 2009. From the 480 stool samples collected, 300 originated from alligators of a closed farming system (150 hactchlings, 80 grow out phase and 70 breeding animals), while 180 originated from alligators of an open system (100 grow out and 80 breeding animals). The samples were analyzed for fecal floating (Willis-Mollay method) and simple sedimentation (Lutz method), according to methodology described by Hoffman (1987) and observed by optical microscopy. The results showed oocysts of the following genera *Eimeria* and *Isospora* and eggs of *Acanthostomum*, *Balantidium*, *Capillaria*, *Dujardinascaris* and *Strongyloides*. Statistically, a higher degree of parasitism by coccidia was observed in both systems studied. In conclusion, the parasitism found in commercially raised animals occurs due to factors such as stress and feeding; and parasitism may occur through breeders from breeding regions as well.

KEY-WORDS: Commercial farming. Qualitative diagnosis. Willis-Mollay method. Lutz method.

RESUMO

O objetivo desta pesquisa foi realizar um diagnóstico qualitativo dos gêneros de parasitos encontrados em amostras fecais de jacarés (*Caiman latirostris* Daudin, 1802) criados comercialmente (sistema *farming* de criação), no período de 2008 a 2009, no estado do Rio de Janeiro. Inicialmente foram colhidas 480 amostras, sendo 300 de criatório comercial de sistema fechado (150 filhotes, 80 engorda e 70 reprodução) e 180 de criatório comercial de sistema aberto (100 engorda e 80 reprodução). Em seguida as amostras foram submetidas a análises coproparasitológicas de flutuação (método de Willis-Mollay) e de sedimentação simples (método de Lutz), segundo metodologia descrita por Hoffmann (1987) e visualizadas por microscopia óptica. Os resultados obtidos evidenciaram a presença de oocistos dos gêneros *Eimeria* e *Isospora* e ovos de *Acanthostomum*, *Balantidium*, *Capillaria*, *Dujardinascaris* e *Strongyloides*. Ao final do experimento foi possível concluir que o parasitismo encontrado em animais de criatórios ocorre devido a fatores como pressão de estresse e alimentação e que a transmissão parasitária pode ocorrer através de matrizes e reprodutores oriundos de regiões nascedouras ou intracriatórias.

PALAVRAS-CHAVE: Criação comercial. Diagnóstico qualitativo. Método de Willis-Mollay. Método de Lutz.

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INTRODUCTION

Alligators, in general, are opportunistic predators that feed on any live or dead animal, including the same species if the potential prey is smaller, especially hatchlings (SANTOS et al., 1993). Waste product such as poultry, fish and pork are the best option for feeding alligators in captivity. However, because they are offered raw, the alligators run the risk of bacterial, fungal or parasitic infections (CATTO, 1991; REBECA et al., 1991; VERDADE, 2004).

Some alligators, as the *Caiman latirostris* (Daudin, 1802) show a considerable economic potential. Thus, there is great interest in commercial farming of the species in Brazil (CATTO, 1991; VERDADE, 2001). Romanelli et al. (2002) states that the crocodile as a whole can be commercially used, nothing is wated. The meat can be found in several establishments in Brazil and according to records of specialized legal restaurants, in 1999, consumption was approximately 30 tons, at a price of about $10.00 per kilogram (ROMANELLI et al., 2002). Non-conventional cuts, such as trunk and limbs, can be used to produce smoked or canned sausages and burgers. Viscera are being studied to produce meat meal (ROMANELLI & SCHMIDT, 2003). The leather has wide acceptance in the international market (VERDADE, 2004). Since the U.S. opened its market to alligator skin, other countries were encouraged to do the same. According to Coutinho (2008) only this market is valued at about $200 million annually. Legalized commercial farming, in addition to ensure a regular supply of meat and leather to the consumer market and to create a new income for Brazil, will help to preserve the species.

There are few studies reported in the literature on the subject, and almost all are restricted to species description, without information on the structure of supra-community, host-parasite interactions and environment, prevalence and infection intensity. Therefore, the aim of this study was to conduct a qualitative diagnosis of the genera of parasites found in fecal samples from alligators (*C. latirostris*) commercialized in Rio de Janeiro, during the period 2008-2009.

MATERIAL AND METHODS

This study was approved by the Ethical Committee of Animal Use of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, under protocol number 058/2009-CEUA/UENF. All ethical principles for animal experimentation adopted by the Brazilian Society of Animal Science Laboratory/Colégio Brasileiro de Experimentação Animal have been observed.

The commercial farm, located in the state of Rio de Janeiro, had a closed system breeding tanks, whose females were captured in their natural environment in the Pantanal region of Mato Grosso do Sul. In this system, the alligators had free access to water and soil and veterinary assistance as well. Sanitary measures such as foot baths and truck disinfection were also provided, and these commercial farms were located far from urban areas.

Hatchlings were placed in closed tanks that worked as nursery, where temperature and humidity were closely monitored and ranged from 30 to 37ºC and above 80%, respectively. While breeding animals remained in open tanks with available vegetation to simulate the natural habitat of the species. One male and two females were placed in each open tank. The animals were classified according to their age, hatchlings from 1 to 12 months old, grow out phase between 12 and 24 months, and breeders above 24 months.

Feeding took place every 15 or 30 days depending on digestion and age class as well, and consisted of scraps of poultry, fish and pork from slaughterhouses that were considered improper for human consumption. Water from the tank, which was also drinking water, was changed twice during the year.

In the closed tanks to simulate the natural environment there were other animals, such as turtles and fish, which were also found in the open tanks together with regional birds, small rodents and other reptiles. The human presence, either for treatment or feeding, is also part of the environment of alligators kept in captivity.

Fecal sampling took place where the alligators basked in the sun in order to minimize stress and ensure the safety of those involved in the task. Sampling was conducted according to protocol established by Hoffmann (1987), where only the tops and interiors are collected, in order to prevent contamination. Samples were then placed in stool collection containers duly identified and kept in a cooler at 4ºC. Samples were transported to Animal Health Laboratory, in the Parasitology Department of the Veterinary Hospital, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF).

Each sample was analyzed qualitatively following the Willis-Mollay fluctuation method and simple sedimentation (Lutz method), according to methodology described by Hoffmann (1987). Subsequently, eggs and oocysts were observed, diagnosed and the genera determined by optical microscopy (Bioval® or Quimis®) under magnifications 40X, 100X and 400X (HOFFMANN, 1987).

The results were analyzed statistically by the chi-square (χ^2) test (VIEIRA, 1998).

RESULTS

Parasites were found in 48.33% (145/300) and 13.88% (25/180) of the samples collected in the closed and open system tanks, respectively. Coccidia were the most frequent in both systems (Table 1). The overall statistical analysis showed that the presence of endoparasites was not related to age class in either system, closed or open ($P \geq 0.05$, $\chi^2 = 1.10$; $\chi^2 = 0.23$, respectively) (Table 2). However, the analysis of each genus showed a relationship of parasitism with age class and the system used.

Table 1 – Numbers of total and infected samples for different age classes and genus, in the period 2008 - 2009, in a commercial farm (open and closed systems) of *Caiman latirostris.*

Age classes	samples total	samples infected	genus	eggs/oocysts
Closed system				
Hatchlings (0-12 mo.)	150	75	*Eimeria* sp.$^{+++}$	50
			Isospora sp.$^{+++}$	70
			Balantidium sp.$^{+}$	1
Grow out (12-24 mo.)	80	40	*Eimeria* sp.$^{+++}$	14
			Isospora sp.$^{+++}$	35
			Balantidium sp.$^{+}$	1
Breeders (>24 mo.)	70	30	*Eimeria* sp.$^{+++}$	10
			Isospora sp.$^{++}$	18
			Acanthostomum sp.$^{+}$	1
			Dujardinascaris sp.$^{+}$	1
Total (%)	300 (100,00%)	145 (48,33%)		
Open system				
Grow out (12-24 mo.)	100	15	*Eimeria* sp.$^{++}$	5
			Isospora sp.$^{+}$	15
			Capillaria sp.$^{+}$	3
			Strongyloides sp.$^{+}$	3
Breeders (>24 mo.)	80	10	*Eimeria* sp.$^{+}$	3
			Isospora sp.$^{+}$	1
			Capillaria sp.$^{+}$	1
			Strongyloides sp.$^{+}$	5
Total (%)	180 (100,00%)	25 (13,88%)		

$^{+}$ < 5 eggs/oocysts per field
$^{++}$ between 5 and 10 eggs/oocysts per field
$^{+++}$ >10 eggs/oocysts per field

Table 2 – Results of the chi-square test for fecal samples of alligators (*Caiman latirostris*) raised in commercial farms according to age classes, after parasitological tests.

Age classes	Parasitological tests Positive	Parasitological tests Negative	Total
Closed system			
Hatchlings (0-12 mo.)	75	75	150
Grow out (12-24 mo.)	40	40	80
Breeders (>24 mo.)	30	40	70
Total	145	155	300
χ^2 = 1,10; P≥0,05			
Open system			
Grow out (12-24 mo.)	15	85	100
Breeders (>24 mo.)	10	70	80
Total	25	155	180

χ^2 = 0,23; P≥0,05

Eimeria was found in the closed system tanks and its presence was related to age class ($P \leq 0.01$, $\chi^2 = 12.33$). A relationship that was not observed in the open system tanks ($P \geq 0,05$, $\chi^2 = 0,16$) (Table 3). On the other hand, *Isospora* was related to age class in both systems, open and closed ($P \leq 0.05$; $P \leq 0.01$, $\chi^2 = 9.00$; $\chi^2 = 10.38$, respectively) (Table 4).

Due to low counts obtained for endoparasites of the genera *Balantidium* sp., *Acanthostomum* sp. and *Dujardinascaris* sp., the statistical analysis was not possible for the samples collected in the closed system tanks.

The presence of the endoparasites *Capillaria* sp. and *Strongyloides* sp. in fecal samples collected in the open tanks was not related to age class ($P \geq 0,05$, $\chi^2 = 0,63$; $\chi^2 = 1,11$, respectively) (Table 5).

DISCUSSION

Alligators (*Caiman* sp.) are naturally opportunist predators, that is, any small animal such as birds, rodents, reptiles or mammals, nearby can become prey (POOLEY, 1989; PINHEIRO & LAVORENTI, 2001). This predatory practice while feasible in open tanks where preys have free access, is not feasible in tanks of the closed system. Since alligators are exclusively carnivores, they become subject to a large variety of parasites when they capture and ingest practically whole preys (LAVORENTI & PINHEIRO, 2001). However, in commercial farms, alligators in captivity are fed poultry, fish, pork and beef scraps or meat from slaughter houses refused for human consumption, which increases the risk of gastrointestinal parasitism of captive animals (LAVORENTI & PINHEIRO, 2001; PINHEIRO & LAVORENTI, 2001; MULLER et al., 2005). In addition, many species of flies can carry eggs and larvae of helminths, thus becoming potential vectors in case blood sucking species attacks the alligators (FERREIRA et al., 2002; OLIVEIRA et al., 2002).

Few studies in the literature focus on the coproparasitological diagnosis of alligators, which even though are tough and extremely resistant, may experience gastrointestinal parasitism as evidenced in this study. The fact that animals, most of the time, do not have symptoms of gastrointestinal parasitism does not exclude the possibility that they are behaving as hosts, albeit paratenic (GILIOLI & SILVA, 2000; BATISTA, 2006).

The occurrence of positive results for oocysts of *Isospora* sp. and *Eimeria* sp. in all tanks where stool samples were collected, and because these genera have already been detected in samples of alligators belonging to 11 different zoos in São Paulo state, suggests that this type of parasitism is relevant among animals in closed areas (GILIOLI & SILVA, 2000).

The reptiles are fed outside the water, the food is thrown near the edges of the tanks and ingested in large pieces since they are not chewed, just torn. Thus, remainder food debris and stool may attract other species to the tanks, especially wild birds, abundant in the region, which may be parasitized by *Eimeria* sp. and *Isospora* sp. (ADRIANO et al., 2000; FREITAS et al., 2003; DAUGSCHIES et al., 2004; DOLEZALOVA et al., 2004; MARQUES et al., 2007; GOMES, 2009).

An important factor to consider is that open commercial tanks, home to breeder animals, are located near forest areas, without any protection to prevent the entrance of other animals that can carry and spread parasites (GILIOLI & SILVA, 2000).

Also striking is the fact that *Eimeria* sp. and *Isospora* sp. were apparently more prevalent among the alligators reared in the closed system tanks, where monitored temperature and humidity were kept relatively high, in order to accelerate growth and metabolism of these animals, making them more active (PINHEIRO et al., 2000; CAMPOS, 2007). However, in addition to favoring animal growth, these conditions also favor proliferation and dissemination of the parasite oocyst in the ponds. The closed system not only impedes tank exposure to direct sunlight but also promotes super population, which can help direct transmission of parasites among the reptiles (URQUHART et al., 1996).

Regarding the influence of water on the degree of parasitism between the two commercial systems studied, a higher coefficient was found for the closed system, where high temperature in conjunction with feces deposited in the water, enables parasite development.

To help reduce parasites in the site, glass should replace the plastic covering the roof of the closed system to increase sunlight incidence in the ponds. In closed tanks, the presence of fish that feed on alligator feces, helps to keep the environment free or with less waste. On the other hand, these fish can also work as a reservoir for several species of nematodes making them potential transmitter of parasites (URQUHART et al., 1996; FOREYT, 2005; MENEZES et al., 2006).

In this study, coccidia of the genera *Eimeria* and *Isospora* were prevalent in the closed systems, the nursery and grow out ponds. In grow out ponds of the open system, there was a predominance of the genus *Isospora*. These observations suggest that the overcrowded ponds of the closed system, especially the nurseries populated with hatchlings, caused higher parasitism, corroborating results of several authors who investigated the presence of coccidia in feces of animals raised in intensive systems (GENNARI et al., 1999; SEQUEIRA & AMARANTE, 2002; FREITAS et al., 2003; FOREYT, 2005; SILVA et al., 2008; GOMES, 2009). Another kind of parasite diagnosed in the closed system commercial nursery and also in stool samples of hatchlings was *Balantidium*, an intestinal parasite present in human and pigs, which has already been observed in reptiles (turtles and tortoises), birds and wild pigs (MUNDIN et al., 2004; FOREYT, 2005; TITTOTO et al., 2005). Tittoto et al. (2005) diagnosed *Balantidium* sp. in fecal samples of turtles raised in captivity.

Carmo & Salgado (2003) identified the presence of cysts of this parasite in the water of primates in the reserve area of the Mariano Procópio Museum, in Juiz de Fora, MG, thus demonstrating that contaminated

Table 3 - Results of the chi-square test for fecal samples of alligators (*Caiman latirostris*) raised in commercial farms according to age classes, after parasitological tests for *Eimeria* sp.

Age classes	Parasitological tests		Total
	Positive	Negative	
Closed system			
Hatchlings (0-12 mo.)	50	100	150
Grow out (12-24 mo.)	14	66	80
Breeders (>24 mo.)	10	60	70
Total	74	226	300

$\chi^2 = 12{,}33$; $P \leq 0{,}01$

Open system			
Grow out (12-24 mo.)	5	95	100
Breeders (>24 mo.)	3	77	80
Total	8	172	180

$\chi^2 = 0{,}16$; $P \geq 0{,}05$

Table 4 - Results of the chi-square test for fecal samples of alligators (*Caiman latirostris*) raised in commercial farms according to age classes, after parasitological tests for *Isospora* sp.

Age classes	Parasitological tests		Total
	Positive	Negative	
Closed system			
Hatchlings (0-12 mo.)	70	80	150
Grow out (12-24 mo.)	35	45	80
Breeders (>24 mo.)	18	52	70
Total	123	177	300

$\chi^2 = 9{,}00$; $P \leq 0{,}05$

Open system			
Grow out (12-24 mo.)	15	85	100
Breeders (>24 mo.)	1	79	80
Total	16	164	180

$\chi^2 = 10{,}38$; $P \leq 0{,}01$

Table 5 - Results of the chi-square test for fecal samples of alligators (*Caiman latirostris*) raised in commercial farms according to age classes, after parasitological tests for *Capillaria* sp. and *Strongyloides* sp.

Age classes	Parasitological tests		Total
	Positive	Negative	
Capillaria sp.			
Grow out (12-24 mo.)	3	97	100
Breeders (>24 mo.)	1	79	80
Total	4	176	180

χ^2 = 0,63; P≥0,05

Strongyloides sp.			
Grow out (12-24 mo.)	3	97	100
Breeders (>24 mo.)	5	75	80
Total	8	172	180

χ^2 = 1,11; P≥0,05

water may serve as an infection agent of this parasite. Once the food offered to the alligators is contaminated by this parasite, the water of the pond can also be contaminated. *Balantidium* genus was also diagnosed by the methods of Hoffmann and Willis-Mollay in fecal samples of free and zoo birds, thus showing the potential of this parasite to infect different hosts, and indicating that the birds are also possible hosts of this genus (GOMES, 2009). Their presence detected only among animals populating closed tanks suggests an infection transmitted by eating contaminated meat from pork or poultry.

Infection by the genus *Strongyloides*, which contains approximately 40 species that can infect several animals such as birds, amphibians and mammals (UENO & LENGY, 1998; CARMO & SALGADO, 2003), was diagnosed only in small taxas and in animals from open tanks, and may be occurring through the ingestion of wild birds that came into the tanks to search for food and/or water (AMARANTE & OLIVEIRA, 2002; OLIVEIRA et al., 2002; MULLER et al., 2005; MARRA et al., 2007). This hypothesis is reinforced by the fact that animals raised in the closed system were not infect with *Strongyloides* sp.

Mismanagement, poor water quality and hygiene, since men are also hosts of these parasites, and the fact that the open system ponds are located far from farm headquarters and have no adequate sanitation, should all be factored in when studying parasitism of commercially reared alligators (KOBAYASHI et al., 1995; LUNA et al., 2007; BACHUR et al., 2008). Another possibility for dissemination of *Strongyloides* sp eggs is the mechanical transmission by flies, since helminth eggs and larvae have already been observed adhered to fly body and inside the gut, thus facilitating contamination of the local, food and even the animals themselves (D'ALMEIDA et al., 2002; FERREIRA et al., 2002; OLIVEIRA et al., 2002).

The genus *Acanthostomum,* a trematode that has already been identified parasitizing the intestine of alligators and crocodiles, was diagnosed in one closed system pond populated with breeders (MORAVEC, 2001; FERNANDES et al., 2002; TKACH & SNYDER, 2003; FOREYT, 2005). According to the work of Gomes et al. (2000), parasites of this genus were found in the intestines of silver catfish (Jundia), a freshwater fish found in Central and South America. Corrêa & Brasil-Sato (2008) diagnosed *Acanthostomum* sp. in Surubim, a fish from the Sao Francisco Basin in the state of Minas Gerais, thus demonstrating that this genus is recurrent in Brazil and these fish may be fed to the alligators.

The genus *Dujardinascaris* was diagnosed in this study in samples taken from the closed breeding tank. According to Foreyt (2005), this genus has already been identified in the guts and stomach of alligators and crocodiles. The same was observed by Moravec (2001) while studying crocodiles of Central America. Lakshimi & Sudha (2000) identified this genus in the intestines of mullet, a fish well-known and used in our country, suggesting infection of crocodiles that feed on these fish.

Capillaria is another genus identified in this study in samples collected in open system grow out and breeding ponds. Some studies show that this genus is present in caracarás, field quails, dogs, cats, chickens, among other species (URQUHART et al., 1996; KNOFF et al., 2006; MARTIN et al., 2006; GOMES, 2009). Their presence has also been reported in bats and amphibians from South America, which shows a great potential to infect several species (IANNACONE, 2003; ROJAS & GUERRERO, 2007). According to Santos et al. (1985), these parasites were also found in fecal examinations of the Indian tribes of nearby Guaporé and Mamoré rivers in Rondonia. As this type of parasite is highly resistant, it can infect a multitude of hosts, and since it was diagnosed in the samples from open commercial ponds, it is possible that the infection occurred through the alligator predation of wild birds and/or small rodents, or contamination of the place and food by the flies that can act as the infection vector of this parasite (D'ALMEIDA et al., 2002; FERREIRA et al., 2002; OLIVEIRA et al., 2002).

CONCLUSIONS

The parasites found in the commercial alligator farm, according to the current study, are due to two factors: stress and feeding. However, parasite transmission can occur through matrices and breeders that came from other hatching areas (Pantanal/MS, Jacarepaguá/RJ, etc.), where they could have been infected by a series of parasites or even during transportation. Regarding infections in the rearing farm, several intrinsic and extrinsic factors are related among them, food availability (animal carcasses, scraps, viscera and water), predation (relationship predator/prey) and improper management (strategic feeding, presence of animals, vectors and man, water exchange, control of the environment and super population as well). The high incidence of coccidia especially seen in poultry reared in commercial closed farms, underlines the stress factor, which is intensified when associated with overcrowding.

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