UNIVERSIDADE VILA VELHA - ES

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DE ECOSSISTEMAS

ONE WORLD, ONE HEALTH: BROAD-SNOTED CAIMAN (*Caiman latirostris*) AS SENTINELS OF ECOSYSTEM HEALTH

YHURI CARDOSO NÓBREGA

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Catalogação na publicação elaborada pela Biblioteca Central / UVV-ES

N754o Nóbrega, Yhuri Cardoso.
One world, one health : broad-snoted caiman (*Caiman latirostris*) as sentinels of ecosystem health / Yhuri Cardoso Nóbrega . - 2021.
71 f. : il.

Orientador: Carlos Eduardo Tadokoro.
Tese (Doutorado em Ecologia de Ecossistemas) - Universidade Vila Velha, 2021.
Inclui bibliografias.

1. Ecologia. 2. Biologia - Conservação. 3. Jacarés. I. Tadokoro, Carlos Eduardo. II. Universidade Vila Velha. III. Título.

CDD 577

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DEDICATÓRIA

Dedico este trabalho à família minha Família, em especial aos meus pais, à mãe terra e a todos os animais que nela habitam.

.

AGRADECIMENTOS

À Deus

Primeiramente agradeço a Deus, senhor e criador da natureza. Muito obrigado por tudo que tem me concedido e por me dar cada dia mais ânimo, determinação e foco para lutar pela conservação da biodiversidade. Muito obrigado por me confiar a mais nobre das missões: cuidar da criação, utilizando a ciência para promover a harmonia na relação homem-natureza.

À minha família

À Rita, Fábio, Yasmin, Babi, Lolla, Bugui, Lupita e demais membros da família Cardoso- Nóbrega, deixo registrado meu amor incondicional. É difícil transcrever em palavras o tamanho de minha gratidão por vocês. Sem vocês, nada seria possível. Muito obrigado por todos os sacrifícios pessoais que têm feito ao longo destes anos para que eu possa ser cada vez melhor como pessoa e profissional. Muito obrigado por compreenderem minha ausência constante (momentos durante os quais estava participando de atividades para conservação da natureza). Muito obrigado por tudo! Amo vocês de forma incondicional!

Aos meus pais

Deixo um registrado um agradecimento especial aos meus pais. É impossível descrever o tamanho do meu amor e minha gratidão por TUDO e POR TANTO !! VENCEMOS !! VENCEMOS JUNTOS !! Graças aos infinitos sacrifícios que vocês fizeram ao longo de tantos anos de nossas vidas !! Esse título é de vocês !! Se hoje eu sou médico veterinário, mestre e doutor em ciências, devo absolutamente TUDO A VOCÊS!! Muito obrigado por cada sacrifício, cada gesto de amor, e principalmente por me ensinar sobre o AMOR, ALEGRIA, HOMBRIDADE e NATUREZA. Nenhuma universidade no mundo teria a capacidade de me ensinar tanto como vocês me ensinaram !! Se hoje podemos fazer a diferença para a proteção da natureza, é graças a vocês! Esse título é dedicado à Fábio Nóbrega de Souza e Rita de Cassia Cardoso

Nóbrega de Souza, DOUTORES DO AMOR E SABEDORIA!! AMO VOCÊS DO TAMANHO DO INFINITO.

Ao Instituto Marcos Daniel (IMD)

Ao IMD meu eterno amor e gratidão.

À ArcelorMittal Tubarão

Parceiros de longa data para as atividades que visam à conservação dos jacarés no Brasil, muito obrigado por acreditarem na importância do trabalho desenvolvido e pelo apoio financeiro.

Ao Projeto Caiman – Jacarés da Mata Atlântica

Missão de vida, a partir da qual, junto com uma equipe fantástica, temos conseguido fazer a diferença, contribuir escrever história na conservação da biodiversidade no Brasil;

Ao Instituto Últimos Refúgios

Pela parceria de sempre em nossas empreitadas pela conservação dos jacarés e seus ambientes naturais no Brasil.

Aos amigos de pós-graduação

Aos meus amigos, gratidão por sempre estarem ao meu lado ao longo desta jornada.

Ao Prof. Dr. Carlos Eduardo Tadokoro

Gratidão por cada ensinamento e por tantos momentos marcantes ao longo desta jornada.

***Aos membros da banca**

Aos grandes professores e pesquisadores, membros da banca de avaliação, deixo registrado meu muito obrigado a cada um de vocês por dispor do seu valoroso tempo para que este trabalho seja engrandecido.

À Natureza

Deixo registrado meu amor incondicional à natureza. Estarei sempre dedicando minha vida à sua proteção. Salve a mãe terra! Somos todos um só, somos todos biodiversidade.

Gratidão!

SUMÁRIO

RESUMO

CARDOSO NÓBREGA, YHURI, D.Sc, Universidade Vila Velha – ES, Junho de 2021. **ONE WORLD, ONE HEALTH: BROAD-SNOTED CAIMAN (Caiman latirostris) AS SENTINELS OF ECOSISTEM HEALTH.** Orientador: Dr Carlos Eduardo Tadokoro

Dada a atual degradação na Mata Atlântica, bioma que possui maior densidade demográfica do país, as relações eco-epidemiológicas dos ambientes tem se alterado, e iniciativas que visem à compreensão dos aspectos que envolvem a ecologia das enfermidades e sua relação com as comunidades e ecossistemas devem ser consideradas prioridade para que se possa alcançar a conservação dos ambientes naturais, bem como dos serviços ecossistêmicos. A proximidade dos remanescentes de Mata Atlântica com grandes centros urbanos oferece condições que influenciam de forma direta a relação saúde-doença da fauna silvestre, como dispersão de patógenos, proliferação de vetores, surgimento de animais sinantrópicos e exposição da fauna a disruptores endócrinos. Neste contexto o C. latirostris possui grande relevância, uma vez que é uma espécie generalista e ocorre tanto em ambientes impactados, quanto em ambientes naturais, sendo assim a compreensão da ecologia de doenças e ecoimunologia desta espécie representa um grande avanço para se compreender a relação entre os impactos ambientais e a ocorrência de enfermidade em animais silvestres.Este projeto objetiva avaliar a saúde e compreender quais patógenos (parasitos e microrganismos) ocorrem nas populações de *Caiman latirostris in situ* e *ex situ*, bem como suas relações com a saúde única. Para realizar o projeto serão amostradas diferentes áreas na Mata Atlântica com diferentes níveis de impacto antrópico. Foram amostrados jacarés jovens, juvenis e adultos em cada ambiente, na qual passaram por avaliação clínica e exames laboratoriais para determinar o perfil microbiológico e parasitológico das populações estudadas. Os resultados obtidos nesta tese, indicam que o jacaré-de-papo-amarelo é uma espécie sentinela da saúde ambiental e possui relação direta com os aspectos macrosanitarios de OneHealth. Os dados obtidos nesta tese representa um grande passo para o conhecimento da saúde e diversidade de patógenos em ambientes aquáticos impactados pelas atividades humanas. Tal conhecimento é essencial para o desenvolvimento de políticas públicas e ações para a conservação das espécies de crocodilianos no Brasil.

Palavras chaves: Ecologia, biologia da conservação, crocodilianos, ecologia de doenças, herpetologia, saúde única, medicina da conservação.

ABSTRACT

CARDOSO NÓBREGA, YHURI, D.Sc, University Vila Velha - ES, June 2021. **ONE WORLD, ONE HEALTH: BROAD-SNOTED CAIMAN (Caiman latirostris) AS SENTINELS OF ECOSISTEM HEALTH.** Advasor: Dr Carlos Eduardo Tadokoro

Given the current degradation in the Atlantic Forest, a biome that has the highest demographic density in the country, the eco-epidemiological relationships of environments have been changing, and initiatives aimed at understanding the aspects that involve the ecology of diseases and their relationship with communities and ecosystems. they must be considered a priority in order to achieve the conservation of natural environments, as well as ecosystem services. The proximity of the remnants of the Atlantic Forest to large urban centers offers conditions that directly influence the health-disease relationship of wild fauna, such as dispersion of pathogens, proliferation of vectors, emergence of synanthropic animals and exposure of fauna to endocrine disruptors. In this context, *C. latirostris* has great relevance, since it is a generalist species and occurs in both impacted and natural environments, so understanding the ecology of diseases and ecoimmunology of this species represents a major advance to understand the relationship between environmental impacts and the occurrence of disease in wild animals. This project aims to assess health and understand which pathogens (parasites and microorganisms) occur in the populations of *Caiman latirostris in situ* and *ex situ*, as well as their relationship with one health. To carry out the project, different areas in the Atlantic Forest with different levels of anthropic impact will be sampled. Young, juvenile and adult caimans were sampled in each environment, in which they underwent clinical evaluation and laboratory tests to determine the microbiological and parasitological profile of the studied populations. The results obtained in this thesis indicate that the broad-snoted caimans is a sentinel species of environmental health and has a direct relationship with the macro-sanitary aspects of OneHealth. The data obtained in this thesis represents a major step towards understanding the health and diversity of pathogens in aquatic environments impacted by human activities. Such knowledge is essential for the development of public policies and actions for the conservation of crocodilian species in Brazil.

Keywords: Ecology, conservation biology, crocodilians, disease ecology, herpetology, one health, conservation medicine.

INTRODUÇÃO GERAL

A Mata Atlântica é um dos ambientes mais biodivervos e ameaçados do planeta, sendo uma das áreas prioritárias para a conservação (Silva, 2005). Dentre as ameaças à conservação de vertebrados neste bioma, destacam-se a perda de habitat em decorrência da fragmentação, a contaminação da fauna por disruptores endócrinos, a caça e a introdução de espécies exóticas (Mittermeir et al., 2004).

Dada a atual degradação das áreas naturais no Brasil, especialmente na Mata Atlântica, bioma que possui maior densidade demográfica do país, iniciativas que visem à conservação de espécies e ecossistemas devem ser consideradas prioridade para que se possa alcançar o desenvolvimento sustentável e assim garantir a conservação dos ambientes naturais, bem como os serviços ecossistêmicos (Silva, 2005). Estudos sobre ecologia e saúde da fauna silvestre são ferramentas importantes para a definição de estratégias de conservação para subsidiar políticas públicas para conservação de espécies ameaçadas (Primack & Rodrigues, 2001; Silva, 2005; Mangini & Silva, 2006).

A proximidade dos remanescentes de Mata Atlântica com grandes centros urbanos pode oferecer condições que influenciam de forma direta a relação saúde-doença da fauna silvestre, como dispersão de patógenos, proliferação de vetores, surgimento de animais sinantrópicos e exposição da fauna a disruptores endócrinos (Silva, 2005). A análise criteriosa da relação entre saúde animal, saúde humana e saúde ambiental auxilia na compreensão das relações eco-epidemiológicas e assim na determinação de estratégias de ação que visem à manutenção da saúde e da homeostasia dos ecossistemas (Silva, 2005). Estes são pressupostos da biologia da conservação que propõe uma abordagem ampla dos problemas ambientais, sociais e de saúde por uma ótica transdisciplinar e integrada (Gillin et al., 2002).

Patógenos são parte da comunidade biológica e desempenham um importante papel no equilíbrio dinâmico das populações de seus hospedeiros em especial, na manutenção de seu status imunológico (Mangini et al., 2012; Mangini & Silva, 2006). Porém, situações extremas nas quais as doenças se tornam vetores de extinção devem ser investigadas pois geralmente estão associadas a fatores antrópicos, indicando desequilíbrio ambiental. Algumas doenças tem sido investigadas devido a sua influência na diminuição populacional e aumento da vulnerabilidade a extinção de diversas espécies de vertebrados, complicando ainda mais o cenário antropogênico de perda de biodiversidade em que o planeta se encontra (Mangini et al., 2012). Algumas doenças tem se destacado como indicadores de problemas ambientais, enquadrando os seus hospedeiros como espécies sentinelas, incluindo os jacarés (Poletta et al., 2008).

Espécies sentinelas são aquelas que indicam a ocorrência de problemas ambientais antes que estes ocorram de forma visível, geralmente através de problemas de saúde (Mangini & Silva, 2006). Neste contexto o C. latirostris possui grande relevância, uma vez que é uma espécie generalista que ocorre tanto em ambientes alterados, quanto em ambientes naturais. Sendo assim a compreensão da ecologia de doenças desta espécie representa um grande avanço para se compreender a relação entre os impactos ambientais e a ocorrência de enfermidade em animais silvestres (Coutinho et al,, 2013; Mangini & Silva, 2006; Nóbrgea & Santos, 2017).

O jacaré-de-papo-amarelo é considerado um crocodiliano de porte médio que ocorre apenas na América do Sul, em regiões úmidas do Brasil, Argentina, Uruguai, Bolívia e Paraguai (Almonacid et al., 2007; Grommbridge, 1987; Bassetti, 2016) (Imagem 1). Segundo Coutinho (2003), 70% das populações de C. latirostris encontram-se em território brasileiro. No país, a espécie é encontrada no Cerrado, na Caatinga, na Mata Atlântica e nos Pampas, ocupando ambientes lênticos, como lagos, manguezais, brejos e pântanos de água doce ou salobra (Coutinho et al., 2013; Filogonio et al., 2010) (Imagem 2).

Figura 1: Jacaré-de-papo-amarelo (*Caiman latirostris*). Foto: Leonardo Merçon.

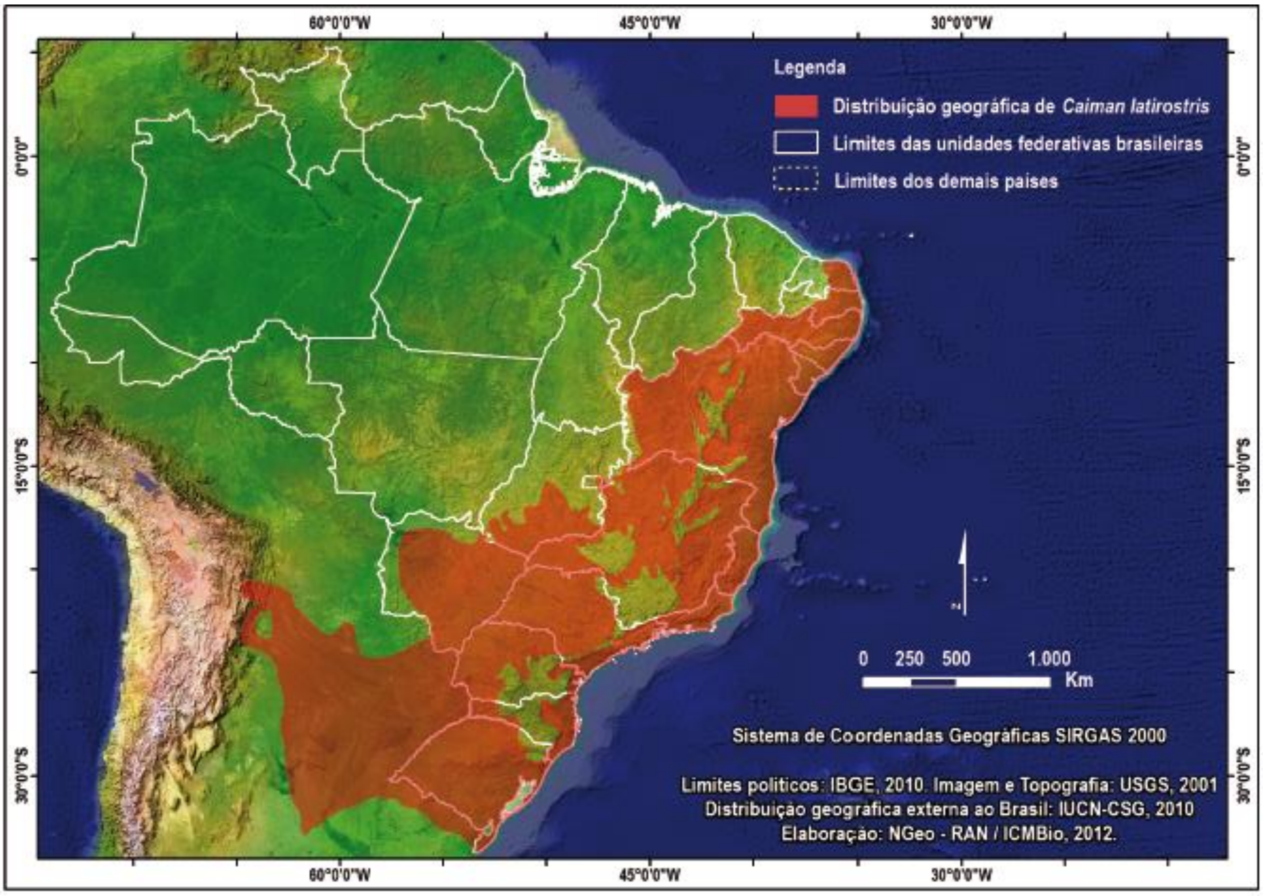

Figura 2: Distribuição geográfica das populações Jacaré-de-papo-amarelo (*Caiman latirostris*). Fonte: Coutinho, et. al, 2013.

As populações de C. latirostris possuem um papel importante no fluxo energético dos ambientes nos quais estão inseridas. São essenciais para a manutenção do equilíbrio do ambiente e de serviços ecossistêmicos, além de serem consideradas espécies sentinelas da saúde dos ecossistemas (Poletta et al., 2008; Coutinho et al., 2013; Filogonio et al., 2010; Bassetti, 2016; Nóbrega, 2017).

O jacaré-de-papo-amarelo possui a situação mais complexa de conservação dentre os crocodilianos que ocorrem no Brasil (Bassetti, 2016; Vilella, 2004).

A poluição dos ambientes aquáticos acarreta desequilíbrio fisiológico nos organismos e causa problemas ecológicos nas populações, além de proporcionar um cenário favorável à ocorrência de doenças infecciosas e parasitárias por comprometerem a imunidade, causando debilidade nas populações e refletindo de forma negativa na saúde do ecossistema (Primack & Rodrigues, 2001; Silva, 2005; Mangini & Silva, 2006). Assim, a compreensão dos aspectos ecológicos, sanitários e ecoepidemiológicos em ambientes com diferentes níveis de impacto antrópico é necessária para se delinear o contexto da conservação de crocodilianos no Brasil (Imagem 3) (Mangini & Silva, 2006; Nóbrega, 2017).

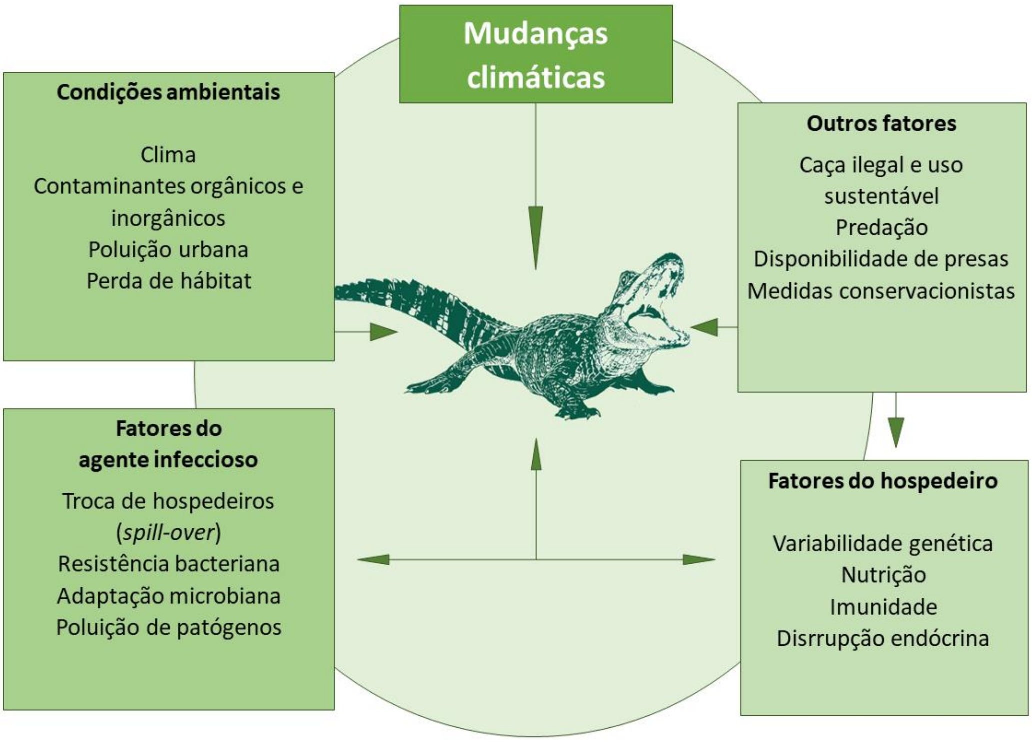

Imagem 3: Ameaças a conservação das populações de Caiman latirostris em um ambiente impactado pelas atividades antrópicas. Fonte: Coutinho, et. al, 2021.

Pouco se sabe a respeito da ecologia dos patógenos que ocorrem no ambiente natural e o real impacto que estes promovem nas populações de crocodilianos no Brasil. Esta carência de informações compromete a definição de programas de conservação adequados que incluam o componente sanitário em suas metas e ações. A avaliação de saúde ou a ocorrência de problemas de saúde nem mesmo é mencionada nas avaliações do status de ameaça de crocodilianos pelos órgãos ambientais brasileiros responsáveis pela conservação de espécies (Coutinho et al., 2013).

O conhecimento da saúde e diversidade de patógenos de Caiman latirostris, em ambientes com diferentes níveis de impacto antrópico, é essencial para se determinar como patógenos se enquadram na ecologia da espécie e como ameaça a sua conservação no Brasil (Nóbrega & Santos, 2017; Nóbrega, 2019).

Neste sentido o presente estudo é norteado pela necessidade de se compreender as doenças e patógenos que ocorrem nas populações de *C. latirostris* in situ e ex situ, bem como suas relações com a saúde única.

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CHAPTER I

BROAD-SNOTED CAIMAN (*Caiman latirostris)* FROM PERI-URBAN ENVORIONMENT CARRY MULTIDRUG-RESUSTANT BACTERIA

Broad-snouted caiman from peri-urban environment carry multidrug-resistant bacteria.

This article was submitted to the scientific journal EcoHealth

Abstract

Habitat loss and expansion of urban areas are pushing *Caiman latirostris* to live in peri-urban environments. It is important to assess the animal health risks and zoonotic potential of this species microbiota for people in contact with caimans. We describe the isolation of bacteria found in cloacas of *C. latirostris* and its resistance to antibiotics. Our results indicate that the presence of the *C. latirostris* in peri-urban environments besides been an ecological issue is also a challenge to public health. Finding multi-resistant bacteria in the autochthone microbiota of wild caimans is a warning about the deterioration of the ecosystem health in peri-urban environments.

Broad-snouted caiman from peri-urban environment carry multidrug-resistant bacteria.

Habitat loss and poaching threaten caimans survival and population's viability in Brazil (Verdade et al., 2010). The expansion of cities over natural environments has altered the distribution of broad-snout caiman, *Caiman latirostris* (Daudin, 1802), bringing them closer to the urban centres and inducing the species to a synanthropic behaviour. Thus, they occupy anthropized and polluted areas, getting exposed to contaminants, injuries and infectious/parasitic diseases (Filogonio et al., 2010; Coutinho et al., 2013). *C. latirostris* can be used as an environmental health sentinel (National Research Council, 1991; Poletta et al., 2008) i.e. a good indicator to understand and prevent disease and intoxication risks, reflecting environmental conditions (Rey et al., 2006). *C. latirostris* synanthropy also exposes humans to contact with the species, allowing the transmission of pathogens to people through the consumption of contaminated meat from poaching or wounds caused by accidents involving crocodilians (Cupul-Magaña et al., 2005), like those reported in other countries (Caldicott et al., 2005; Langley, 2005; Lamarque et al., 2009; Shepherd and Shoff, 2014). Charruau et al. (2012) evaluated the oral and cloacal microbiota of crocodiles in Mexico finding pathogenic bacteria, but they did not evaluate antibiotic resistance.

Caimans are exposed to several contaminants in peri-urban environments, including antibiotics. Under a One Health perspective antibiotic resistance is an important issue that must be addressed by multiple sectors since it is an urgent problem on human, animal and ecosystem health with economic and security implications (White and Hughes 2019). Most antibiotics used to prevent and treat infections in humans and animals are partially metabolized and excreted, going to sewage treatment plants or directly to waters and soils (Dong et al. 2018; Xu et al. 2019). The use of antibiotics in animal production also has a great influence on the generation of bacterial resistance in pigs, poultry, cattle (An et al. 2017) and fish (Manage 2018). In agriculture, antibiotics are topically applied to plants, although in smaller quantities than in human and veterinary medicine and animal production (Heuer et al., 2011). Beyond the indiscriminate use of these drugs, the release of antibiotics contaminated effluents into the environment increases the likelihood of bacteria acquiring resistance to antimicrobials commonly used in human and animal medical routine (Bengtsson-Palme and Larsson, 2015).

The overlapping of caiman's habitats with cities makes it important to know the zoonotic potential of caiman's microbiota. We describe the isolation of aerobic bacteria with zoonotic potential found in cloaca of wild *C. latirostris* from anthropized peri-urban environment and the resistance of these bacteria to antibiotics.

We captured 14 wild caimans in a peri-urban forest environment area of the municipality of Serra, state of Espírito Santo, Brazil (23° 13' 42"S, 40° 15' 05" W). The study area has two lagoons that receive inputs from neighbouring domestic sewage making the environment visually eutrophic and contaminated, with a characteristic sewage smell and high density of macrophytes.

We captured the caimans at night from a small motorboat, using a steel-wire noose and a spotlight to obfuscation. Each animal underwent a clinical evaluation including the parameters: behaviour during capture (active, listless), visual body score (good, medium or bad), coloration of the cloacal mucosa (normal, hypo or hyper-coloured), mucosal capillary filling time and biometrics. The set of assessments was summarized in the apparent health parameter (good, medium or bad). After clinical evaluation, sexing and sample collection were performed. We measured the snout-vent length (SVL) and the weight of animals. The individuals were classified into three size classes based on the SVL: pre-pubertal (<40 cm), pubertal (40–90 cm) and mature (> 90 cm) (Coutinho et al. 2005).We collected the samples using sterile swabs directly from the cloaca and placed in Stuart medium tubes in an isothermal container at 8 ºC and sent to the laboratory within 12 hours for culture. The samples were inoculated into sterile Petri dishes containing blood and MacConkey agar, incubated at 35 °C for 24 to 48 hours. We isolate and identify the bacteria first evaluating macroscopic aspects of the predominant colonies, considering colour, border shape, appearance, elevation and haemolysis capacity and microscopic aspects, such as shape, arrangement and Gram staining (Procop et al. 2017) followed by biochemical colorimetric tests with the equipment Vitec 2 Biomerieux® using specific kits according to the manufacturer´s protocols (Pincus, 2016).

After identification we evaluated the antimicrobial resistance using the disc-diffusion test (CLSI, 2018) with 15 antibiotics: ampicillin, amoxicillin plus clavulanic acid, aztreonam, cefepime, cefotaxime, piperacillin plus tazobactam, amoxicillin, ceftazidime, ciprofloxacin, ceftriaxone, sulfamethoxazole plus trimethoprim, chloramphenicol, tetracycline, cephalothin, cephalexin (Table 1). These antibiotics are between the most commonly used in our veterinary diagnostic routine.

After clinical evaluation, the captured all specimens appeared clinically healthy (good apparent health), the number of males was significantly greater than that of females (12/2), and had an average SVL 180, 9 cm (± 52.41) and an average weight of 30.13 kg (± 21.4).

We identified the following bacteria: *Escherichia coli* (5/14), *Klebsiella pneumoniae* (1/14), *Morganella morganii* (1/14), *Proteus mirabilis* (1/14), *Pseudomonas aeruginosa* (3/14), *Salmonella* spp. (1/14).

We made antibiograms from these six isolates (Table 1) and there was evidence of high antibiotic resistance, especially for *E. coli*, *P. mirabilis* and *M. Morganii*. No resistance was observed in *Salmonella* spp..

As expected, since it was a cloacal microbiota, the Enterobacteriaceae family was prevalent in the samples (*E. coli*, *Salmonella* spp., *E. cloacae*, *M. Morganii*, *P. mirabilis*, *K. pneumoniae*). They are Gram-negative and include an abundant variety of pathogenic bacteria belonging to the natural gastrointestinal microbiota of animals and humans and others found mainly in soil and water (Paterson, 2006).

Opportunistic infections by enterobacteria in the respiratory and urinary tract, and sepsis in hospitalized human patients are frequently reported (Cruz et al., 2007; Amaral et al., 2009; Mehar et al., 2013; Dias et al., 2015). *E. coli*, *Salmonella* spp., *M. Morganii*, *Klebsiella* sp., *Serratia* sp. and *P. mirabilis* have been isolated in cases of sepsis in crocodilians (Huchzermeyer, 2003; Novak and Seigel, 2013). These are zoonotic important bacteria and their ability to produce disease is conditioned to the host immune status or to contact with pathogenic strains, as in the case of *E. coli* (Kaper et al. 2004) and *Salmonella* spp. (Barman et al. 2008), which cause severe infections in healthy individuals, leading to sepsis and death (Behnsen et al. 2015; Giannella et al. 2018). Uhart et al. (2013) reported the presence of *Salmonella infantis* and *Salmonella nottingham* in cloaca of *C. latirostris* in captivity in Argentina, both with capacity to cause disease in healthy humans. Salmonellosis transmitted by reptiles has been frequently reported in humans in the United States and Europe, especially owners of snakes, turtles and lizards kept as pets (Corrente et al., 2017), but little is known about the transmission by crocodilians (Uhart et al., 2011).

Pseudomonas sp. was reported in the oral cavity of *Crocodylus acutus* (Cuvier, 1807) in North America (Cupul-Magaña et al., 2005). *P. aeruginosa*, belongs to the family Pseudomonadaceae and also presents ubiquitous characteristic being considered an opportunistic microorganism with high mutagenicity and resistance to drugs routinely used in the medical routine (Mata and Abegg, 2007). The imbalance of the regular microbiota of animals favours the proliferation and substitution of it by *P. aeruginosa*, which in areas of damaged tissue generates an infection with endotoxin liberation (Diggle and Whiteley 2020).

Except for *Salmonella* spp., all isolates showed antimicrobial resistance. The animals used in the study were wild and never experienced antimicrobial treatment, thus, the observed resistance reflects the presence of resistant bacteria in the environment where they live.

The efforts against the development of resistance to antibiotics was initiated mainly in hospital environments and later in agricultural environments (Gaynes, 1997; Talon, 1999). Antibiotic resistance has been observed in several aquatic environments including domestic sewage, hospital sewage, rivers, sediments, surface waters, lakes, oceans and drinking water systems (Karkman et al., 2016; UNEP, 2017). Development of bacterial resistance is favoured by the exposure of microorganisms to low and sub-lethal concentrations of antibiotic within a matrix (water, soil, sediment, leaves, suspended particles and biofilm), which allows the selection and persistence of frequent mutations in bacteria that are able to grow in the external environment, which is the case of many opportunistic pathogens (Bengtsson-Palme and Larsson, 2016). Nutrient-rich habitats with low concentrations of antibiotics have characteristics for the development of resistant microorganisms (Baquero et al., 2008), as the lagoons in our study area.

Contaminated water is often used for irrigation of agricultural land, recreational practice and as drinking water supply (after further treatment). Domestic animals (pets and cattle) that usually drink untreated surface water and wild animals present in these environments may help in the spread of resistant bacteria (Bengtsson-Palme and Larsson, 2016).

Bacterial resistance is a worldwide concern with ecosystemic and economic effects, posing a challenge for public health (WHO, 2015). It leads to a decrease in treatment alternatives, since infections previously considered easy to treat become resistant to antibiotic therapy, leading to clinical failing in both animals and humans, which can culminate in the death of patients with infections that should be of simple treatment with antibiotic substances (Santos, 2004). Presence of antibiotics in the environment alters the metabolic activity of the microbiota, causing an impact on the structure of the bacterial populations of the environment, and may remain affected even after the dilution and disintegration of the antibiotics in the place (Martinez, 2009) affecting its ecological functions.

The occurrence of multidrug-resistant pathogenic bacteria in the cloacal microbiota of broad-snouted caimans in a peri-urban environment raises a concern about the zoonotic risk, since these microorganisms pose a risk to human health, especially because there is contact and consumption of broad-snouted caimans through poaching in the studied region. We recommend that the treatment of any lesion of caiman bites should be done based on isolation and antibiotic resistance testing to avoid ineffective use of antibiotic therapy. It also raises concern about possible modifications in the microbial ecology of aquatic environments favouring the development of pathogenic microorganisms.

We reinforce the need of a One Health approach, including multisectoral and multidisciplinary control measures that requires health professionals and society to see human health deeply dependent on how we interact not only with our microbiome but also with the animals and the environment.

Ethical Approval

This research was made under the ICMBio Sistema de Autorização e Informação em Biodiversidade - SISBio permit #48537-8 and was approved by the Commission of Ethics on the Use of Animals (CEUA-UVV), Protocol #394-2016, Universidade Vila Velha (UVV), Vila Velha, ES, Brazil.

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Table 1.

Resistance (+) and sensitivity (-) to antimicrobial substances of isolated bacteria from cloaca of wild broad-snouted caimans (*Caiman latirostris*) living in a peri-urban environment (Serra, ES, Brazil).

Antimicrobial substance	*Escherichia coli*	*Klebsiella pneumoniae*	*Morganella morganii*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Salmonella* spp.
Ampicillin	+	+	+	+	-	-
Tetracycline	+	-	+	+	-	-
Amoxicillin	+	-	-	-	-	-
Amoxicillin plus clavulanic acid	+	+	+	+	-	-
Cephalothin	+	-	-	-	-	-
Cephalexin	+	-	-	-	-	-
Ceftriaxone	-	-	+	+	-	-
Cefotaxime	-	-	-	+	-	-
Cefepime	-	-	+	+	+	-
Sulfamethoxazole plus trimethoprim	-	-	+	+	-	-
Piperacillin plus tazobactam	-	-	+	+	-	-
Aztreonam	-	-	+	+	+	-
Chloramphenicol	-	-	+	+	-	-
Ceftazidime	-	-	+	+	+	-
Ciprofloxacin	-	-	-	-	+	-

CHAPTER II

BIOLOGICAL HAZARD ASSOCIATED TO BACTERIA FROM *Caiman latirostris* NESTS AND EGGS

Biological hazard associated to bacteria from *Caiman latirostris* nests and eggs

This article was published in scientific journal Herpetology Notes (Herpetology Notes, volume 12: 905-908, 2019).

Abstract: Research, conservation, production, and other activities that deal with the management of broad-snouted caiman (*Caiman latirostris* Daudin, 1802) expose professionals to biological hazards related to microorganisms that exist in the environment of animals, including some bacteria harmful to human health. Therefore, to improve the knowledge about the bacteria present in the nests and eggs of these caimans is very important to development of strategies of diseases prevention and reduction of the biological risk related to the professional activities. We aimed to study the species of bacteria present in nests and eggs of *C. latirostris*. We performed the identification of the species after growth in culture medium, the species identified were tested for resistance or sensitivity to antimicrobial substances. Our findings may be useful to occupational biosafety programs that should be designed to prevent biological risks and to maintain the health of professionals involved in the management of *C. latirostris*.

The broad-snouted caiman (*Caiman latirostris,* Daudin, 1802) is an important species to keep the homeostasis and health of its environment therefore it is considered a flagship and umbrella species, very important for conservation of South American biodiversity (Nóbrega & Santos, 2017). It is actually classified under low extinction risk by the International Union for Conservation of Nature and Natural Resources (IUCN, 2017) because of the broad geographic distribution, inhabiting rivers, swamps, and mangroves of Bolivia, Brazil, Paraguay, Argentina, and Uruguay, although local extinctions are common in the Atlantic Forest (Coutinho et al., 2013).

In South America, many research, conservation, and production activities involve handling of *C. latirostris* eggs and nests. However, little is known about the potential health risks of this activity to involved professionals. Therefore, to know the bacteria present in these nests and eggs is fundamental for establishing biosafety programs to minimize the occupational biological risks.

The objective of this work was to identify the bacteria present in nests and eggs of *C. latirostris* and test their resistance against antimicrobial agents.

Samples were collected from three different nests found in an enclosure of caimans in a private zoo in state of Espírito Santo, southern Brazil, in the Atlantic forest biome. The enclosure resembles the natural environment and is composed by a lake and an area with vegetation where females can build their nests freely. In each nest, 10 eggs were scrubbed with sterile swabs (Olen®, model K41-0102), and a small sample of nest substrate was collected in a sterile flask. Afterwards each nest sample was placed in sterile saline (NaCl, 0.85%, Êxodo Científica®, Brazil; sterilized at 121º C, 20 min), homogenized for 1 h at 145 RPM (Kline mixer, Nova Técnica®, Brazil), and 1 mL of each sample was inoculated in Cistine-Lactose, Electrolyte Deficient (CLED) Agar plates (Kasvi®). Each swab was placed in sterile saline, and the same procedure was performed but homogenization. All plates were incubated for 48 h, at 37º C, in aerobic conditions (Figure 1). Each bacterial isolate was phenotypically identified by conventional microbiological tests and Bactray® kit: I and II sets for oxidase-negative bacteria; III set for oxidase-positive bacteria (Laborclin, Brazil)

(Procop et al., 2017). Antimicrobial sensitivity tests were performed by disk diffusion test according to the protocols established by the Clinical and Laboratory Standards Institute (CLSI, 2015; CLSI, 2018).

The following bacteria species were identified: (i) from nest samples, *Bacillus cereus, Enterobacter cloacae, Klebsiella ozaenae,* and *Klebsiella pneumoniae*; (ii) from egg samples, *Acinetobacter baumannii-calcoaceticus, Bacillus cereus, K. ozaenae*, *K. pneumoniae*, *Lecrercia adecarboxylata,* and *Pseudomonas aeruginosa* (Table 1). Therefore, nest handlers are indeed in contact with these bacteria and it is important to know how they have been related to human infections.

Bacillus cereus is a Gram-positive mobile rod, soil saprophyte and facultative aerobic bacterium from the Bacillaceae family. When exposed to oxygen, it develops spores that are resistant to dry, high temperatures, ionizing irradiation, and some chemical compounds and it's pathogenicity is associated to food born gastrointestinal intoxications (Kotiranta et al., 2000). However, *B. cereus* pathogenicity as a primary agent of severe non gastrointestinal infection is well documented in humans and frequently under estimated (Bottone, 2010).

Pathogenic bacteria from *Klebsiella* genus are ubiquitous in nature and found in most of natural environments, sometimes in symbiosis with plant roots (Struve & Krogfelt, 2004). However, species *K. pneumoniae, K. oxytoca,* and *K. granulomatis* were already isolated from patients suffering from nosocomial infections (Podschun & Ullmann, 1998) and this intestinal colonization is important for disease transmission (Struve & Krogfelt, 2004). *K. pneumoniae* is a Gram-negative rod, non-sporulated facultative aerobic bacteria. It is easily isolated from oral cavities as saprophytes, which can reach the lungs and cause a pulmonary or urinary infection in immunodeficient patients, with high mortality, specially the hypervirulent clinical variant (Podschun & Ullmann, 1998; Pomakova et al., 2012).

Enterobacter cloacae is an opportunistic infectious agent in hospital environments (Hoffmann & Roggenkamp, 2003). The most frequent *E. cloacae* infections are urinary and respiratory, endocarditis, and septic arthritis (Schlesinger et al., 2005) and conjunctivitis or secondary infections (Tinoco et al., 1997). *E. cloacae* is an infectious agent, highly resistant to antibiotics (around 60%), because of its high capacity to produce broad spectrum beta-lactamase (Soto et al., 2008).

Lecrercia adecarboxylata, former *Escherichia adecarboxylata*, is an opportunistic and mobile Gram-negative rod. It belongs to the Enterobacteriaceae family and has a broad distribution in aquatic environments, food, and human fluids like faeces, urine, sputum, and blood. There were few cases of poly-microbian-opportunistic infections in immunocompromised individuals where this bacterium was found (Keren et al., 2014).

Pseudomonas aeruginosa species, form the Pseudomonadaceae family, is characterized as a Gram-negative rod, strictly aerobic bacterium, which produces toxins and biofilm (Mata & Abegg, 2007). It is found in the normal intestinal human microbiota, but it can also live in water, vegetables, soil, and moistened habitats (Santos & Colombo, 2015). Diseases caused by this bacterium range from local to fulminant sepsis in immunocompetent individuals, but most cases are in between these two sides, as superficial skin infections associated to contact with contaminated water (Palleroni, 2010).

Acinetobacter genus is Gram-negative cocobacillus, sessile, not fermentative, catalase-positive and oxidase-negative. The complex *A. baumannii-calcoaceticus* (*A. calcoaceticus, A. baumannii, A. pitti e A. nosocomialis*) have phenotypically analogue species, among which *A. baumannii* is the most clinically prevalent (Silva, 2009)., As an opportunistic an biofilm maker bacterium, *A. baumannii* is commonly associated to nosocomial infections, surviving in hot and humid environments, therefore colonizing soil, water, animals, and fomites (Fournier et al., 2006). Moreover, this species has a high ability to develop antibiotic resistance, leading to the appearance of multi-resistant strains that cause high mortality indexes (Peleg et al., 2008).

According to what has been described about the bacteria found in nests and eggs of *C. latirostris*, handling these objects can expose people to infectious diseases that can compromise intestinal functions, cause skin lesions, and even death if a combination of specific conditions occur (e.g., an antimicrobial resistant strain infecting an immunocompromised person). Given this risk, it is also important to check if these strains were susceptible to any antibiotic available.

From all tested antimicrobial substances (piperacillin associated to tazobactam, cefepime, imipenem, amikacin, ciprofloxacin, ampicillin, and aztreonam), resistance was only observed to ampicillin in *E. cloacae*, *K. ozaenae* e *K. pneumoniae* isolates. All other bacteria were sensitive to the tested antibiotics (Table 1).

It is known that bacterial resistance is defined as a group of adaptation mechanisms against deleterious or lethal effects to them. When microorganisms have natural resistance to any antimicrobial agent (by a known structure/process), this resistance is called intrinsic resistance (Zhang & Feng, 2016). This resistance is encoded in plasmids, happens when bacteria are under conditions in favour of DNA spontaneous mutations, and can be transmitted to other bacteria strains by conjugation. When inappropriate use of antibiotics occurs, this resistance dissemination is favoured (Antonio et al., 2009). Bacterial resistance is a serious and frequent public health problem, therefore preventive measures are necessary to avoid and identify them, like auditing of antimicrobial agents, hand hygiene, patient interventions, and better information for general people and health agents (Oliveira & Silva, 2008).

Caimans nests are built from available material from vegetation, like sticks and leaves, mixed with soil (Kushlan & Kushlan, 1980; Merchant et al., 2018). Good humidity, temperature, and a high care of nest and incubation are related to low proliferation of infectious agents, high embryo survival, and sex determination (Bassetti, 2007). Extreme conditions, like high humidity and temperature, can increase the chances of fungal and bacterial infections in the nests and, therefore, handlers can be infected when dealing with them. To avoid this, establishment of specific Standard Operating Procedures (SOPs) is highly recommended, as well as the use of Personal Protective Equipment (PPE) (disposable latex or silicon gloves and health care particulate protective mask), correct hygiene of incubators, materials and equipment for nest and eggs transportation, and good quality enclosure conditions (Bassetti, 2007).

Studies of *C. latirostris* in natural habitats involves logistic difficulties, which are not present in studies with captive animals where handling is more frequent, enhancing the occupational risks. On the other hand, traditional communities and biologists who work with caimans tend to avoid the use of SOPs, either out of ignorance or lack of habit, which makes them potential victims of infections. Therefore, both situations demand the awareness of personnel involved about the risk of exposure to pathogenic microorganisms and the need to follow SOPs and use appropriate PPEs.

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Tables

Table 1. Species of bacteria found in nests and eggs of *Caiman latirostris*, resistancy or sensitivity to antimicrobial substances administered (piperacillin associated to tazobactam, cefepime, imipenem, amikacin, ciprofloxacin, ampicillin, and aztreonam). N = nests, E = eggs, S = sensitive, R = resistant.

Species	Where	Ampicillin	Other antimicrobial substances administered
Acinetobacter baumannii-calcoaceticus	E	S	S
Bacillus cereus	N, E	S	S
Enterobacter cloacae	N	R	S
Klebsiella ozaenae	N, E	R	S
Klebsiella pneumoniae	N, E	R	S
Lecrercia adecarboxylata	E	S	S
Pseudomonas aeruginosa	E	S	S

Figures

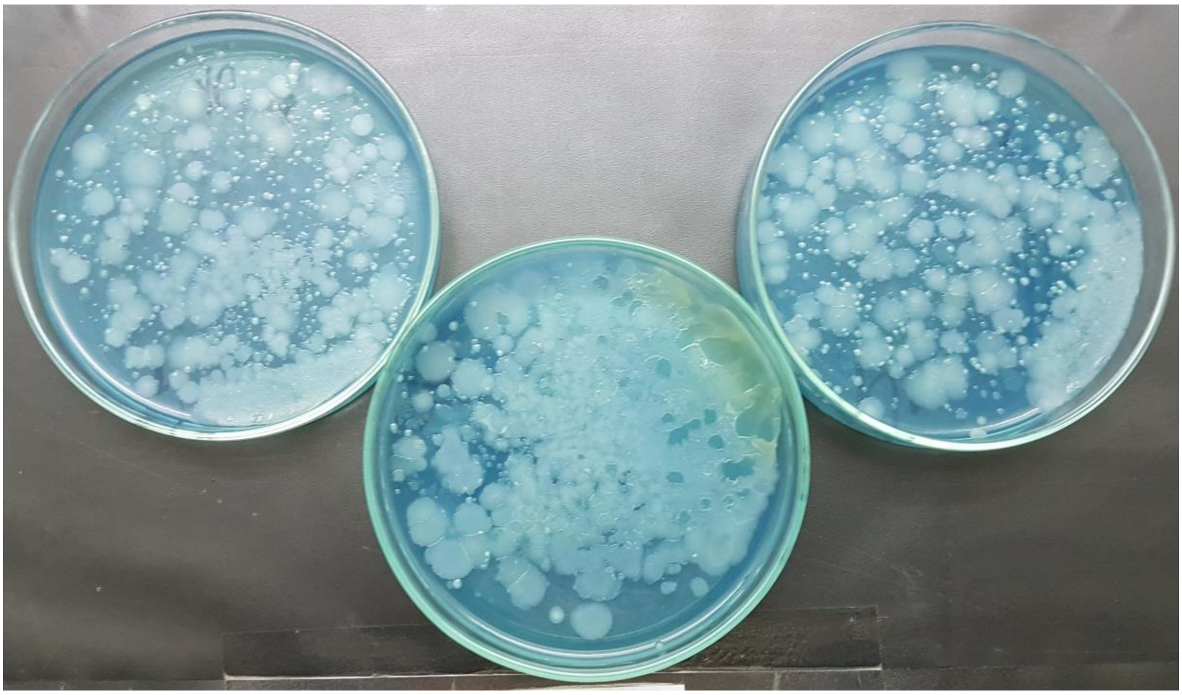

Figure 1. Bacterial culture of nest substrate, 48 h after inoculation, at 37º C. These plates are triplicates of the 10:3 dilution. This result is representative of 3 independent experiments.

CHAPTER III

PREDOMINANT BACTERIAL MICROBIOTA OF THE MOUTH AND CLOACA FROM CAPTIVE *Caiman latirostris* (Daudin, 1802).

Predominant bacterial microbiota of the mouth and cloaca from captive *Caiman latirostris* (Daudin, 1802).

This article was published in scientific journal Herpetology Notes (Herpetology Notes, volume 13: 451-455, 2020)

The broad-snouted caiman, *Caiman latirostris* (Daudin 1802), has a wide geographical distribution throughout a large portion of South America between latitudes 5° N and 34° S in water bodies and wetlands in Brazil, Argentina, Uruguay, Bolivia, and Paraguay (Coutinho et al., 2013). According to the International Union for Conservation of Nature (IUCN), *C. latirostris* faces a low extinction risk and a least concern (LC) conservation status (Crocodile Specialist Group, 2019). *C. latirostris* is a medium-sized crocodilian (up to 3.5 m length) with a generalist carnivorous diet, feeding on crustaceans, insects, fish, amphibians, reptiles and small mammals depending on the life stage (Diefenbach, 1979; Borteiro et al., 2009). This species is kept in captivity at commercial breeding sites as well as conservation and zoological institutions in different parts of the world (Bassetti and Verdade, 2014). Besides indispensable for the ecosystem balance, these animals also have economic and social importance (Carreira et al., 2015).

Brazilian crocodilians are widely exploited and represent an important part of the economy, culture and subsistence in traditional communities (Rieder et al., 2004; Botero-Arias et al., 2010; Nóbrega and Santos, 2017). Consumption, production, research and conservation of caimans usually involve direct handling of specimens. However, little is known about the potential zoonotic risks of this activity related to direct contact with caiman's feces and urine, secretions and blood, bites and other accidents.

In Brazil, the use of *C. latirostris* as resource is generally associated with illegal subsistence poaching and legally regulated farming and industrialization of leather and consumption of meat (Botero-Arias et al., 2010). In illegal trade, both poacher and consumer are exposed to all types of pathogens that may be present in the carcasses, as there are no processing protocols, use of personal protective equipment, controlled disinfection and also there is no surveillance (Viana et al., 2015). Despite the ban on hunting wild animals in Brazil from 1967 (Brazilian Lawnº 5.197, 01 March 1967), the illegal caiman meat trade has been an economic alternative for many traditional populations and is considered an important source of income for these communities (Marioni, Mühlen, and Silveira, 2006).

Extensively farmed wild species such as caimans can be major carriers of pathogens, especially because they live in the aquatic environment, since water is a remarkable vehicle for the transmission of microorganisms. (Oliveira et al., 2015; Santos and Colombo, 2015).

Accidents involving specimens of Brazilian caimans causing serious bacterial infections in humans have already been described in the literature, being considered high-risk injuries by health teams (Deslandes et al., 2008; Neto et al., 2013). Wildlife researchers are also exposed to pathogens when handling caimans or its nests and processing biological samples, common tasks for environmental and epidemiological research and surveillance (Lemos and D'Andrea, 2014; Nóbrega et al., 2019).

In commercial caiman breeding sites, most skin and mucosal injuries result from aggressive behavior among animals, caused by stress in high density pens or reproductive period. During disputes for females or territory, the inoculation of pathogens occurs through the bite, resulting in up to 5% of the mortality and up to 100% of the skin damages, causing economic losses in leather production (Verdade and Santiago, 1992; Ramos et al., 1992).

Studies related to the microbiota of the mouth and cloaca of C. latirostris have been previously developed with the objective of describing the pathogenic bacterial diversity, in order to characterize it and establish appropriate therapeutic approaches to fight infections involving these microorganisms (Ramos et al., 1992; Silva et al., 2009).

Opportunistic infections with bacterial agents from the Enterobacteriaceae family are frequently reported in humans and animals. As opportunistic agents, enterobacteria are generally associated with primary or secondary infections in immunosuppressed patients (Mandell et al., 2005; Santos, 2007; Soto et al., 2008; Clifford et al., 2012; Freitas and Pinto, 2018). In humans, we can highlight fever, diarrhea, meningitis, dermatitis, osteomyelitis (Mandell et al., 2005; Oliveira Souza et al., 2016; Freitas and Pinto, 2018), pulmonary, urinary and respiratory infections that can progress to septicemia caused by enterobacteria (Santos, 2007; Soto et al., 2008; Clifford et al., 2012; Nascimento and Araújo, 2013). This bacterial group is of economic importance for breeding programs in captivity, both for caimans and human health. In addition to representing more than 80% of Gram-negative bacteria isolated in human and veterinary medical routine, some strains of enterobacteria are also considered enteropathogens because they preferentially cause gastrointestinal infections, such as *Escherichia coli* and some *Salmonella spp*. (ANVISA, 2004). They are common in nosocomial infections and have a great ability to survive in hot and humid environments. In addition, they have a high capacity to develop resistance to antibiotics, generating resistant strains and high mortality rates (Lavagnoli et al., 2017).

It is necessary to understand the risks imposed by microorganisms from the mucosa of *C. latirostris* to the establishment of biosafety programs aimed at professionals who deal directly with caimans and education programs aimed at the population. The objective of this study was to describe the most abundant bacteria isolated from the mouth and cloaca of *C. latirostris* from captivity contributing to establish an adequate sanitary conduct in cases of accident involving these animals and humans.

In March 2017 (end of summer), we captured 15 apparently healthy adult specimens of *C. latirostris* in a private zoo located in the municipality of Cachoeiro de Itapemirim, Espírito Santo State, Brazil (20° 53’ 10” S, 41°05’ 56" W). The caiman’s enclosure has a total area of 2.500 m^2 and is composed of a lake with constant natural water renewal, thermoregulation and nesting areas with tree cover for shading. Each enclosure had an average of 40 animals, all pubescent, fed with pieces of muscle, viscera and chicken bones directly in the water. The caimans were captured during the day (8 am to 5 pm), using a steel-wire noose attached to a flexible rod. Then, the animals were manually immobilized, and the mouth was kept closed with adhesive tape around the snout (Bassetti and Verdade, 2014). Sampling was carried out during the day, as the animals were buried in the mud, making it difficult to capture them at night. After restraint, the samples were collected using sterile swabs directly from the cloaca and mouth and put into transport tubes containing Stuart medium, stored in isothermal container at 4 to 8 ºC and sent within 12 hours to the Marcos Daniel Laboratory, in Vitoria, ES. There, the samples were inoculated in Petri dishes containing blood and McConkey agar, incubated at 37 ºC for 24 to 48 hours, according to bacterial growth. Bacterial isolation and identification were made according to macroscopic aspects of the predominant colonies, considering colour, border shape, appearance, elevation, haemolysis capacity and microscopic aspects such as arrangement, shape and Gram staining, followed by colorimetric biochemical testing with the equipment Vitec 2 Biomerieux® (Procop et al., 2016).

The results of the isolates were compiled in Table 1, identifying the collected individual, sample type and the species of bacteria identified. All samples showed bacterial growth. We isolated 10 bacteria species from the cloaca and 7 from the mouth samples.

Bacteria of the Enterobacteriaceae family were isolated in all samples, showing, as expected, the predominance of this family in the microbiota. The most prevalent bacteria in the mouth was *Klebsiella oxytoca* (6; 46%) followed by *Hafnia alvei* (2; 15%), and the other isolates were found in only one sample each. In the cloaca samples the most prevalent isolate was *E. coli* and *K. oxytoca* (4; 21%), followed by *K. ozaenae* (3; 16%), *H. alvei* (2;11%) and other isolates in one sample each.

Enterobacteriaceae was expected to be predominant in the samples based on similar studies (Ramos et al., 1992; Uhart et al., 2011; Silva et al., 2009). Ramos et al. (1992) described the oral microbiota of 19 *C. latirostris*. Enterobacteria of the genera *Escherichia, Proteus, Morganella, Serratia*, in addition to non-sugar fermenting bacteria such as *Pseudomonas* and *Acinetobacter* were predominant, similarly to our result. However, since our work identified only the predominant colonies, Ramos et al. (1992) also identified Gram-positive bacteria of the genera *Streptococcus*, *Staphylococcus* and *Bacillus*, Gram-negative bacteria of the genera *Citrobacter, Providencia, Edwardsiella* and non-fermenter of the genus *Aeromonas*.

Silva et al. (2009) obtained a different bacteria species composition from mouth and cloaca of *C. latirostris* from a zoo in Paraíba, northeast of Brazil, but similarly with 73% of Enterobacteriaceae. The authors suggested that the prevalence of the Enterobacteriaceae family in the mouth mucosa of captive *C. latirostris* may be related to contamination by faeces of the water in the tank in which they live, since the animals spend most of their time underwater. Cupul-Magaña et al. (2005) isolated *Klebsiella pneumoniae, E. coli* and *Pseudomonas sp*. from the mouth of *Crocodylus acutus* emphasizing the pathogenic peculiarity of the oral microbiota and stating that an injury caused by its bite may be capable of causing serious infections in humans. Besides the serious traumatic injuries that caiman bites can cause in humans, important secondary infections requiring the use of antibiotic therapy as described in the case report by Neto et al. (2013). An adult male man who was fishing on the banks of the Paraguay River was attacked in the hand by a Pantanal caiman (*Caiman yacare*) and had to be admitted for treatment of bacterial infection.

Commensal bacteria found in the mucosa of crocodilians can be pathogenic for their hosts when their health is compromised. Novak and Seigel (1986) demonstrate cases of sepsis in American alligator (*Alligator mississippiensi*s), caused by six species of the Enterobacteriaceae family (*Citrobacter freundii, Enterobacter agglomerans, Proteus sp., Morganella morganii, Serratia marcescens* and *Klebsiella oxytoca*), while enterobacteria like *E. coli, Salmonella spp., M. morganii, Klebsiella sp., Serratia sp.* and *Proteus mirabilis* have also been isolated in cases of septicaemia in crocodilians (Novak and Seigel, 1986; Huchzermeyer, 2003). The isolation of the genus *Pseudomonas* sp. has been reported in oral cavity of healthy *C. acutus* in North America (Cupul-Magaña et al., 2005). The imbalance of the normal microbiota of animals promotes its proliferation and replacement by *Pseudomonas aeruginosa*, which in areas of injured tissue generates an infection with endotoxin release (Hirsh and Zee, 2003).

Maintaining captive caimans, whether for conservation purposes, commercial breeding or exhibitions, requires well-designed biosecurity protocols. The health of those who handle these animals is a fundamental issue for public health, since animal confinement environments, although safe, can still be considered a point of transmission of zoonoses. (Viana et al., 2015). Management and nursery protocols are available and must be followed in any situation when health risks are present (Manolis and Webb, 2016). Our data indicate that handling and consumption of *C. latirostris* poses a health risk to professionals and consumers when there is no hygiene or biosafety controls and supervision of breeding and slaughterhouses.

The need for biosafety programs for professionals who carry out this type of activity is unquestionable. Although it sounds obvious, it is still a problem in many places, not only in the undeveloped world. Caimans are poached by traditional communities but people in areas around cities where caiman populations thrive are also specially exposed to low hygiene consumption of caiman meat.

Our data are in line with most studies already published on microbiology of the crocodilians mouth and cloaca. We emphasize that the results presented here are a local characteristic and that different environments will certainly have a different microbiological profile.

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Table 1. Bacterial species isolated from the cloaca and mouth of *C. latirostris* from a wildlife maintainer located in Cachoeiro de Itapemirim, Espírito Santo, Brazil.

Specimen	Sample type	Bacteria identified
1	Cloaca	*Hafnia alvei*
	Mouth	*Klebsiella oxytoca*
2	Cloaca	*Klebsiella oxytoca* *Morganella morganii*
3	Cloaca	*Klebsiella oxytoca*
	Mouth	*Klebsiella oxytoca* *Serratia odorífera*
4	Cloaca	*Lelliottia amnigena*
	Mouth	*Klebsiella oxytoca*
5	Cloaca	*Klebsiella oxytoca*
	Mouth	*Hafnia alvei* *Klebsiella oxytoca*
6	Cloaca	*Hafnia alvei* *Pseudomonas aeruginosa*
	Mouth	*Klebsiella oxytoca*
7	Cloaca	*Klebsiella oxytoca*
	Mouth	*Hafnia alvei*
8	Cloaca	*Escherichia coli*
	Mouth	*Klebsiella oxytoca*
9	Cloaca	*Proteus vulgaris*
	Mouth	*Klebsiella pneumoniae* *Klebsiella aerogenes*
10	Cloaca	*Klebsiella aerogenes* *Escherichia coli*
11	Cloaca	*Klebsiellaozaenae*
12	Cloaca	*Escherichia coli* *Salmonella enterica*
	Mouth	*Acinetobacter baumannii/calcoaceticus* *Enterobacter cloacae*
13	Cloaca	*Escherichia coli* *Klebsiella ozaenae*
14		
15	Cloaca	*Klebsiella ozaenae*

CHAPTER IV

FIRST RECORD OF Amblyomma rotundatum, Koch, 1844 (Acari: Ixodidae) PARASITIZING WILD *Caiman latirostris* (Reptilia: Crocodylidae) IN ATLANTIC RAINFOREST BIOME, SOUTHEASTERN BRAZIL.

First record of *Amblyomma rotundatum* Koch, 1844 (Acari: Ixodidae) parasitizing wild *Caiman latirostris* (Reptilia: Crocodylidae) in the Atlantic rainforest biome, Southeastern Brazil

This article was published in scientific journal Herpetology Notes (Herpetology Notes, volume 12: 9-11. 2019)

Ticks are ectoparasites of great epidemiological importance due to their deleterious action on animal and human health. Direct damage is caused by hematophagy, what also makes ticks as vectors of an impressive number of pathogens (Guglielmone et al., 2003; 2014). While there are numerous records of different tick species infesting Chelonia and Squamata, reports of tick parasitism on South American crocodilians have been limited to a few studies on caimans in Brazil, which included the ticks *Amblyomma dissimile* Koch, 1844*, Amblyomma geayi* Neumann, 1899*, Amblyomma humerale* Koch, 1844 and *Amblyomma rotundatum* Koch, 1844 on *Caiman* spp. and *Paleosuchus* spp. in the Amazon biome (Labruna et al. 2005, Witter et al. 2016, Gianizella et al. 2018), and *A. rotundatum* on *Paleosuchus palpebrosus* Cuvier, 1807 in the Pantanal biome (Morais et al. 2010).

Caimans (Alligatoridae) is the only family of Crocodylia found in Brazil, and *Caiman latirostris* Daudin, 1802 plays an important role as a top predator in the ecological balance in fresh water ecossystems (Nóbrega and Santos, 2017). Currently, more than 70% of the remaining population of *C. latirostris* is found in Brazil, in the Atlantic rainforest, Caatinga, Cerrado, and Pampas Biomes (Coutinho et al., 2013). In this study, we report *A. rotundatum* parasitizing wild *C. latirostris* in the

Atlantic rainforest biome, where tick infestations on caimans have never been reported.

During the year of 2017, 132 specimens of *C. latirostris* were evaluated for the presence of ticks in three different areas of Atlantic rainforest in the state of Espírito Santo, in the municipalities of Serra (-20.225502°S, -40.259538°W), where 115 individuals were captured; Sooretama (-19.059714°S, -39.983408°W), 9 individuals; and Vitória (-20.257760°S, 40.283481°W), 8 individuals. Ticks were preserved in 70% alcohol and identified in a stereomicroscope using taxonomic keys (Aragão and Fonseca, 1961; Guimarães et al., 2001; Barros-Battesti et al., 2006), and later deposited in the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (CNC) of the Faculty of Veterinary Medicine of the University of São Paulo, under the vouchers CNC-3660, 3683 and 3684. Out of 132 specimens, 4 (3%) were parasitized by ticks (Figs. 1 and 2): 1 in Sooretama, 1 in Vitória, and 2 in Serra. A total of 5 tick specimens were collected, all identified as *A. rotundatum* females. At least 3 ticks were fully engorged, indicating that the tick was already attached to the host for several weeks.

This study provides the first report of ticks on *C. latirostris* from the Atlantic rainforest biome, where this crocodilian species has been seriously threated due to habitat loss (Coutinho et al., 2013). The geographic distribution of *C. latirostris* is superimposed with urban areas of greatest population density and large industrial poles in Brazil (Coutinho et al., 2013). This scenario is favorable to the occurrence of infectious and parasitic diseases (Mangini and Silva, 2006), which could indeed include tick infestations. It has been reported that under natural conditions, ticks habitually do not feed on crocodiles (Huchzermeyer, 2003). Indeed, this condition is linked to the strict association of crocodiles to water and the vegetation-free lands surrounding water collections, since ticks are typically associated to vegetation-covered lands (Sonenshine and Roe, 2014). Here, we report 3% of 132 *C. latirostris* individuals infested by *A. rotundatum.* While this tick species has been previously reported on other crocodilian species, namely *Paleosuchus trigonatus* and *Paleosuchus palpebrosus* (Morais et al., 2010, Witter et al., 2016), it is typically associated with terrestrial Squamata and toads of the genus *Rhinella* (Guglielmone and Nava, 2010). The present findings, 'accidental' at a first sight, could be associated to more terrestrial movements of *C. latirostris*. The anthropogenic role on these terrestrial movements, and consequently, on a greater exposure of crocodiles to ticks, should be investigated in further studies. Finally, because the extant crocodile species probably did no coevolve with ticks, the impact of ticks and tick-borne pathogens on crocodiles should also be evaluated.

Acknowledgements. We are grateful to the logistic support provided by ArcelorMittal Tubarão, and Prefeitura Municipal de Vitória, Espírito Santo.

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Figures

Figure 1. A young specimen of *Caiman latirostris* parasitized by a *Amblyomma rotundatum* female.

Figure 2. An adult specimen of *Caiman latirostris* parasitized by a *Amblyomma rotundatum* female.

CONSIDERAÇÕES FINAIS

Uma nova perspectiva relacionada à conservação da biodiversidade tem ganhado terreno nas últimas duas últimas décadas. O conceito de saúde única (*One World – One Health*), criado pela *Wildlife Conservation Society* em 2004, no documento "Princípios de Manhatan", define a saúde como a inter-relação indissociável entre as saúdes humana, animal e saúde ambiental (Zinsstag, et. al, 2011). Na prática, a saúde única implica na adoção de abordagens interdisciplinares na análise, planejamento e execução de ações relacionadas à saúde pública, saúde animal, vegetal e ambiental. Essa abordagem vem sendo adotada pelos organismos internacionais como a Organização Mundial de Saúde (OMS), Organização Pan-americana de Saúde (OPAS) e no Brasil, vem sendo impulsionada pela Fundação Oswaldo Cruz (FIOCRUZ), Conselho Federal de Medicina Veterinária (CFMV) entre outras entidades.

O início do Século XXI vem sendo marcado por diversas pandemias zoonóticas, como é o caso da atual doença mortal COVID-19, causada pelo novo coronavírus (SARS COV-2), com claras evidências que indicam terem surgido ou sido amplificadas por ações antrópicas como o mal uso da terra, consumo e comércio irregular e irresponsável da fauna silvestre, poluição de patógenos e o acelerado e constante trânsito global de pessoas e de produtos de origem animal. Para vencer esses desafios à saúde, garantindo a integridade biológica da Terra para as gerações futuras, são necessárias abordagens interdisciplinares e intersetoriais para educação, prevenção, vigilância sanitária e fiscalização efetiva, monitoramento, controle e mitigação de doenças, bem como para a conservação ambiental de modo amplo (Fao, 2008).

No contexto da Saúde Única, a compreensão do papel ecológico das espécies traz informações importantes sobre a dinâmica das doenças no ambiente e como cada espécie participa do ciclo biológico dos patógenos, como hospedeiro, facilitador, reservatório ou como diluidor dos riscos de surgimento de surtos zoonóticos e doenças que ameaçam a conservação da biodiversidade e consequentemente a saúde ambiental (Nóbrega, 2017; Nóbrega et. al, 2019).

Crocolianos, devido a sua história natural, assumem importância como reservatório de diferentes enfermidades, zoonoses como por exemplo a salmonelose, comum em répteis (Sakaguchi, et. al, 2017), ou como indicadores biológicos de contaminações diversas por contaminantes orgânicos (Poletta et al., 2008), metais pesados (Rainwater et al., 2007) e antibióticos (Nóbrega et al., 2019). Estudos com *C. latirostris* tem demonstrado a utilidade da espécie como bioindicadora de poluição ambiental, através da avaliação dos níveis de contaminantes e como espécie sentinela, através da avaliação dos efeitos destes sobre a saúde das populações (Poletta, Simonello e Mudry, 2016; Nóbrega, 2017; Nóbrega, et. al, 2019).

Desde 1980, estudos utilizam os crocodilianos como bioindicadores e sentinelas da saúde ambiental em relação aos metais pesados dispersos nos ambientes (Manolis, et. al, 2002). Desde então, diversos estudos tem sido desenvolvidos no mundo utilizando diferentes espécies de crocodilianos como sentinelas e bioindicadora da qualidade e saúde ambiental, abordando aspectos sanitários e ambientais.

Na realidade brasileira, o jacaré sobrevive de maneira resiliente em ambientes impactados pelas atividades antrópicas sob diferentes aspectos, sendo assim, torna-se uma espécie fundamental para a avaliação de impactos ambientais e a saúde única dos ambientes onde ocorrem (Nóbrega et. al, 2019; Santos, 2019).

Os resultados do presente estudo corroboram com os demais, consolidando o jacaré-de-papo-amarelo (*Caiman latirostris*) como uma importante espécie no monitoramento saúde única. Portanto, além dos argumentos tradicionais para a conservação dessas espécies como seu valor ecológico e de existência intrínseca, há a questão cultural e socioeconômica, além do valor que legitima a importância dos crocodilianos no contexto de crise de saúde ecossistêmica.

O jacaré, como sentinela, está sujeito a diversos estressores ambientais antrópicos ou naturais, o que torna a avaliação de parâmetros isolados pouco esclarecedora da complexidade dos fenômenos, nos quais as espécies estão submetidas. Dessa forma, um programas de conservação e de monitoramento dos jacarés, como sentinelas, deverá lançar mão de um conjunto de parâmetros de saúde dos indivíduos que possam ser extrapolados para a população e, consequentemente, refletir o papel ecológico da espécie no seu ecossistema.

Ainda há muito a se pesquisar sobre a utilização de crocodilianos como sentinelas da saúde dos ecossistemas na qual estão inseridos. Esta tese consolida a utilização do Caiman latirostris como uma importante espécie sentinela e bioindicadora de impactos ambientais e recomenda a sua utilização em programas de monitoramento de impactos antrópicos sobre os ambientes naturais (Aquáticos e terrestres). Além do uso de *Caiman latirostris* como espécie bandeira para programas de conservação e políticas públicas visando a saúde única e a conservação da biodiversidade brasileira.

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