Summary

The Orinoco caimán (*Crocodylus intermedius*) is a Venezuela own specie, it is seriusly threatened with extinction, because that it have been include in the International Union for Conservation of Nature (UICN) oficial list of critical endangered species. For this motive it has been necessary various efforts of conservation, arduous task due to lack of scientific information about this reptiles. Thus, with big relevance they appear the hemoparasites, what cause morbility and mortality in the same. This research has been made with the object of determine the hemoparasites presence in youngs Orinoco caimán in the zoofarm masaguaral livestock farm. Guarico state. The results from the decriptive stadistics show a estimated hemoparasites presence in the 51 % of the sampled animals with one year of age. For other side, the evidence found in the samples was: Microfilaria, coccoid intraerythrocytic inclusions and in morula shape compatible with Plasmodium spp. and Hepatozoon spp. Also, it was not found significant correlation of the hemoparasites prensence with the variables: Weight, length and body condition. This investigation show diferences with other researchs inherent to the study subject, since there are not microfilariae reports in Orinoco caimans in Venezuela, opening a space for future researchs, the microfilaria hemoparasites in caimans are not desired since the filaries migration in the caimán body can be to increasing the morbility of this, being indicated the euthanasia in the positive animals, so we could will find survival rates really low in wildlife, doing the efforts for Orinoco caimán conservation almost null.

Main

The crocodyles are members of the order Crocodylia, the 28 species and subspecies are divided in 4 subfamilies: Alligatorinae, Crocodylinae, Tomistominae y Gavialinae. The crocodylia order members are found in the better part of tropical áreas around the world. They are adapated to amphibian environments due to their aerodinamics bodys and vertically compressed tail (Mader, 1996). Worldwide study about this prehistoric reptiles which can be found both in sweet wáter systems like in saltwáter systems.

All alive crocodiles include two species of alligators, six of caimans, fourteen of crocodiles and one of gharial, they live less than 4.200 kilometres of the ecuador (Lemmens y Arnold, 2017).

Venezuela is between the 10 megadiverse countries of the world (Díaz, 2000) occupying the sixth place, however Colombia being very similary in it is geography and climates occupies the biodiversity second place. The reason for this diference lies very propably in the lack of scientific information, because over the years Venezuela has been dedicated little time and little efforts to investigation about biologic diversity, except somes institutions and organizations that have been dedicated to the conservation investigation, what has meant the stagnation in terms to obtaining of valuable information about venezuelan flora and fauna, limiting and hindering the actions for diferents species conservation.

In Venezuela the *Crocodylus intermedius* it is know like Orinoco Caiman even though it belongs to the family *Crocodylidae,* the real crocodiles(Barrios, 2019) and it is one of larger native reptiles, as well as one of the four species bigest of the family in the world (Antelo *et al*, 2010), and the only one of them whose distribution is restricted to a watershed: the one from the Orinoco river (Medem, 1981). *C. intermedius* was included in the official list of endangered species according to decree Nº 1486 of 10/09/1996, it is classified for the International Union for Conservation of Nature (UICN) under the category in critical danger (CR) and has come to be considered one of 12 vertebrate species most vulnerable of the world, due to threats like adult hunt, nest looting and destruction of their habitat for river channels contamination or modification: in similar situation are other species of big crocodiles in the world, sharing between them a moderate information about their biology among others aspects, increase knowledge about the specie will contribute for conservation actions.

The first efforts in Venezuela for the Orinoco Caiman studie and consevation date from de decade of 70s, however from 1984 it was established, for iniciative of Tomas Blohm the first conservation program of Orinoco Caiman in masaguaral livestock farm located in Corozopando, Guarico state, being a pioneer in this field to national and international level (Thorbjarnarson y Blohm, 1986) Since then the masaguaral livestock farm has released anually and uninterruptedly a total of 4.040 caimans, representing the 37,5% of the total released to national level, and in the year 2000 increase their scientifics and handling capabilities when established a handling agreement with the Foundation for the Development of Physical, Mathematical and Natural Sciences (FUDECI). From 1990 the environment and renewable natural resources ministry organized the national program for the Orinoco caimán conservation with the participation of several zoofarms of public and private administration (Babarro, 2008), that after 30 years has achieved the released of 10.690 youngs alligators in several rivers of importance inside of distribution área of the specie.

The youngs alligators that are released come from eggs incubation and raising, and young alligators collected in wildlife, as well as those obtained like product of adults mating kept in captivity in zoofarms. This alligators are raised until reaching an ideal size of 80 cm that allows them a survival greater possibility when they are returned to their natural environment. This process is of big importance due to the great dangers that face the young alligators in wildlife, they are natural prey of several animals like spectacled alligator (*Caiman crocodylus*) smuggler wolf (*Tupinambis teguixin*), giant otters (*Pteronura brasiliensis*), cheriway (*Caracara cheriway*) and piranhas (*Pygocentrus cariba, Serrasalmus altuvei)* among others, as well as can also be presented cannibalism among the same youngs alligators and even more serious, nest looting by humans (Antelo *et al.*, 2010).

In the zoofarm this process occurs in specialized lagoons and water tanks, trying to minimize the posible the mortality and the events that may cause alterations in the development of the specimens, to be able to maximize the number of viable specimens that serve for contribute in the maintenance of stable populations in wildlife, however to the date, there is still relatively little information about this animals health, being the most important contributions those reflected by Boede and Sogbe (Boede y Sogbe, 2000). In this sense should be noted that the health monitoring in the specimens that are raised for the nature reintroduction is of big importance because it will allow success of the specimens once released and avoid possible impacts on wild populations from the sanitary point of view.

Under captive conditions, the specimens are susceptible to one series of variables like high densities, unbalanced diets, high incidence of vectors and hygiene bad conditions, among other, that can lead to stress and inmunosuppression situations, making them suceptibles of different diseases development, in particular front to pathogens (Boede y Sogbe, 2000). One pathogenic agents group little studied in the Crocodrylia order are the hemoparasites, that although they have been described in adult specimens without pathogenicity signs, in inmunosuppression and stress situations can cause circulatory problems, hepatics, as well as functionals in the organs where is lodge the parasite in the young caimans, increasing their morbidity and mortality rate, and even affecting the females subsequent fertility (Enríquez *et al.*, 2014). A big number of parasites can be found in peripheral blood in reptiles, among these are included haemogregarines found in blood cells. The parasites can be intracellular or are free in plasma and all require invertebrates like intermediate hosts. The full life cycle of many of this parasites are not know, as well as neither their pathogenicity (Mader, 1996).

The most important parasites that can be present in reptiles are the hemogregarinas to which the gender *Haemogregarina, Hepatozoon, Karyolyscs, Schellackia* y *Lainsonia* belongs and particularly in crocodrilians, the most relevant are *Haemogregarina* y *Hepatozoon.* This can affect various tissues like spleen, liver, kidney and digestive tract, but their blood presence is the way of detection and most important diagnostic, which is done through the blood smears study, where can be found parasites both at the intracellular and extracellular level, like cytoplasmic vacuoles both at the erythrocytes and leukocytes (Enríquez *et al*, 2014). Others parasites like *Trypanosoma* gender like nematodes of filarias group (Order Spirurida, Superfamily Filarioidea) whose larval stage can travel through the bloodstream.

The exposed envidence the big importance of hemotropic agents evaluation in the specimens that will be destined for released in order to guarantee one longer survival without risking to wild individuals, thus allowing one more effective work in populations stabilization for specimens conservation. Because of this, it is necessary determine the hemoparasites presence in the age groups more susceptible like are the young caimans, in one population destined to released, this population is specifically in masaguaral livestock farm, state Guarico, zoofarm center, dedicated in more of 36 years to the Orinoco Caiman conservation, chasing like this with the releases the population stabilization.

In the present investigation, was carry out the determination of hemoparasites presence in youngs Orinoco caimans (*Crocodylus intermedius*) in the zoofarm of masaguaral livestock farm, state Guarico, and for that, blood samples were taken to a number of 87 juvenile specimens. All obtained data was analyzed and described by means of decriptive statistics.

HEMOPARASITES IN CAIMANS EVALUATION

The results of decriptive analysis indicated in the picture 1 Indicated a punctual hemoparasites indicence in the 51% of cases with microfilaria (annex 3) coccoid intraerythrocytic inclusions or in morula shape compatible with Plasmodium spp. and Hepatozoon spp and others (annex 4) while the 49 % of samples were negative. This results show clear evidence that in half of Orinoco caimáns of one year of age exist the hemoparasites presence what makes neccesary further researchs for obtain more hemoparasites information and identify them truthfully, as well as make evident the hemoparasites incidence in caimans and like that can predict a survival rate in wildlife.

When the animals were externally examinated it was evident that the 92 % of the zoofarm animals did not present ticks, defects or major injuries (graphic 1), that show a external health very good and proper handling, it should be noted that one of microfilaria vectors does not leave any identifiable injurie.

PICTURE 1. Percentage of animals with or without evidence of hemoparasites.

|  |  |  |  |
| --- | --- | --- | --- |
|  | EVIDENCE TYPE | FREQ | % |
| HEMOPARASITES PRESENCE TO MICROSCOPE | Negative. | 43 | 49 |
| intraerythrocytic inclusions. | 11 | 13 |
| Coccoids intraerythrocytic inclusions or in morula shape. | 5 | 6 |
| Microfilaria. | 28 | 32 |

GRAPHIC 1.Frequencies distributions of external observations results in the animals

Weight and hemoparasites relationship

Weight descriptive analysis, length to base to tail, total length and body condition, using averages, typical desviations, as well as mínimums and máximums as presented in the picture 2 yielded weight values between 175 y 1.080 gr with averages of 495±199 gr, indicating with this a high varibility in weight ranges, probably caused for the populution high densities in water tanks that cause discrepancies in the weights due to intrapoblational competitiveness for food. For other side the length to base to tail presented variations between 210 and 538 cm with a average of 290 ±55, that represents a acceptable variation, whale the total length presented values more or less stables between 415 y 727 cm with average of 548 ± 72 cm**.**

When was the body condition considered, values were obtained that ranging from 1 to 3 in the scale of 1 to 5 with central tendence (median) of 2, that indicated a body condition of regular to bad due to diet changes and irregularities in the food administration.

PICTURE 2 general description of weight (gr) tail length, total length (cm) and body condition (scale) of the caimans of one year of age.

|  |  |  |
| --- | --- | --- |
| **PARAMETER** | **AVERAGE**±D. **TYPICAL** | **MIN-MAX** |
| Weight | **495 ±199** | **175 -1080** |
| Tail length | **290 ±55** | **210 – 538** |
| Total length | **548 ± 72** | **415-727** |
| Body condition | **2 (Median)** | **1 – 3** |

When was cualitative relationship of weight considered, total length and body condition with the presence of hemoparasites evidence, it was observed the proof of Ji-quadrate (χ2) of independence or contingency, no significant relationship was detected (P > 0,05) of any other parameters with the examinated condition, what it mean that the manifestance of the presence of hemoparasites evidences is independent of the weight, length and body condition, that is, that affect in the same way to any body condition, length and weight of the animals agreeing that was evidenced by Campbell (2006), that prove that the hemoparasitos are transmitted for the horizontal way, existing neccesary vectors for the hemoparasites transmission.

Discussion

This investigation show differences with other works inherent to subject of study, since there is not Orinoco caimans microfilaria reports in Venezuela, opening a field for future researchs, exist a event in the Bararida zoological and botanical park, where was presented three (03) filaria cases on spectacled alligator (*caimán crocodrylus*) (Moleiro, 2017) unpublished data. For other side, was detected in Colombia one case of *Oswaldofilaria medemi* n.sp. on smooth front caimán (*Paleosuchus trigonatus)* from Colombia (Marinkelle, 1981).

With respect to the subject of study for this reaserch, was detected that higher percentage to the half of sampled population have hemoparasites, that make relevant a study about this hemoparasites pathophysiology in caimán organism. Although in this investigation was not found evidence that show significative relationship between the hemoparasites and caimans weight and measures, that is to say, the hemoparasites did not affect their biometrics, the hemoparasites can be affecting the caimán internal organs. Since some hemoprasites like *Hepatozoon, Plasmodium y Haemogregarina* can be found in reptiles without producing pathologies, since in captivity environment, the stress lowers the host defense mechanism, and therefore increase the possibility of development of a clinical disease caused by endoparasites. In other situations the parasitic infestation could go unobserved until a’manifest of a clinical condition or mortality (Mader, 1996); the filarias in dogs produce symptomatology in very advanced states, in caimans can be happening the same.

The filarias migration in caimán body can be increasing the morbidity of this, if the circulatory system is affected aneurysms, thrombosis or a blood vessels constipation may appear, if a masive infestation of larvae (Microfilarias) occur that are in the bloodstream and lympathic vessels can lead to capillary blockages that could cause edema, swelling and necrosis (MUTSCHMANN, 2006) the microfilarias in caimans are unwanted hemoparasites, being indicated the euthanasia for positive specimens, even in caims populations ubicated in Australia, so we can find very low survival rates in wildlife, doing the efforts for the conservation almost null.

Some hemoparasites could not be identified, due that was not detected scientific information about the hemoparasites morphology in Orinoco caimans, leading to other researchs with diagnostic methods more specific, like are of the molecular biology for can do more accurate diagnoses.

METHODS

Geographic área.

Masaguaral livestock farm, also known as Masaguaral ranch, was the study place of this investigation, it is found locatizated in the subregión of the central wavy plains according to the venezuelan environment systems (MARNR, 1983) to south of Guarico state, in the Francisco Miranda municipality, its entrance is in the national road number two in the coordinates 8° 33' 57"N - 67° 34' 16"O (UTM 19P 657253E 947145N), between Calabozo and Corozopando to 320 km from Caracas (anex 1). It is a production unit dedicated to beef cattle breeding and biodiversity conservation banning since 1944 hunting and fishing. This livestock farm works as a private wildlife refuge, in which is dedicated to biological biodiversity protection and one of most threatened ecosystems in Venezuela, through low impact livestock production emphasizing to research and scientific dissemination strategies. In its installations works the Orinoco caimán zoofarm known as “Juan Caimán” in honor to its founder Don Tomas Blohm; with a population of more than 300 Orinoco caiman specimens between reproductive couples and young caimans.

The livestock farm vegetation is of transition between areas known as high plain (Calabozo north) and low plain (San Fernando of Apure south), it is have been identified four physiographic units: medano, bank, bajio and estuary. The medanos constitute sandy areas that never get flood and are irregularly distributed. Some of this zones are of savannah with a predominance of grasses, while in others possess bush forests like dense canopy, wich have heights reaching up to 6 meters. The banks don not get flood, but can be found littler water bodys during the rainy season that constitute the bajios and estuarys that usually retain water and are use for wildlife to the end of the dry season. The zones of moderate use (82%) are important for sustainable livestock management where the animal load do not represent a dangerous for wild flora species permanence in this ecosystems, combinated with modern techniques and traditional handling.

POPULATION AND SAMPLE

The population under study is conformed by young specimens of Orinoco caimans of one year of age, the estimated homogeneous population is of 177 individuals of 2019 cohort, of which were sampled 87 specimens in may 2019, for the sample size determination, the central limit theorem was used, with the porpose of determining a representative quantify for the investigation, the procedure generated a sample size of 87 specimens (n=177).

SAMPLING

In the present study was used proportional stratified sampling with application of the correction factor of finite population. This method allows to obtain through elements separation that make up the population in subgroups that represented the same characteristics; have as fundamental trait that can take a decrease in the estimated stándard mistake or of estimator, what allows the the confidence intervals that is calculate is smaller. This results are especially certain if the measurements given under the strata are homogeneous and the stratas are easily identified, for later one simple sample of each of them. With the described method can be applied the folllowing formula: for finite population is known and whose size is less to 30.000 units.

Where:

Z: reliability margin (expressed in standard desviations).

P: probability of the event happen.

Q: probability of the event do not happen (1-P).

e: estimation mistake (máximum permissible mistake).

N: population (universe to investigate).

N-1: correction factor for finitude.

first approximation.

It is known too what the sample size can be corrected using for that the expression:

where:

corrected sample size

2019 cohort

End = 177

Then:

Where:

Confidence levels 95%

So: Z=1,96

P= 0.5

Q= 0.5

E= 1%=E=0.01

After:

MATERIALS

For specimens handling and subjection elastic bands and plastic container cages were used, in addition, for specimens review and morphometry a wood table, two measuring tapes, a scale hanging type and a cord were used, all intruments before metioned were lended by livestock farm Masagural installations.

For taking samples cotton swabs of ALVE Brand impregnated to 70% isopropyl antiseptic alcohol of EL GUARDIAN brand were used, 4 cotton boxes of 25 grams were used and a 500 milliliter bottle of alcohol, 3 milliliter of sodium heparin, in total 115 disposable syringe of 3 ml of SERI'S brand, 120 disposable hypodermic needle of 23 G x 1 1/2” of GAESCA brand were used. Fort the blood smears perfomance a total of 200 microscope slides of SERI'S brand, 50 coverslip slides of B&C brand, a 160 cubic centimeter container of FIXCELL cells fixative, a red wax crayón, 2 microscope slide boxes of 100 units were used.

For samples processing, a total of 50 milliliter of Giemsa and 50 milliliter of deionized wáter of BI-DESTILADA brand were used, 30 milliliter of immersion oil of DIAGNOTEST brand and an optical microscope model LW200 of LW Scientific brand were used. All materials before mentioned were bought with researchers own funding. In the same way for second review of samples an optical microscope provided by Lisandro Alvarado Central-Western University (UCLA), 20 milliliter of hemacolor and an optical microscope provided by the Bararida Zoo and Botanical park were used.

PROCEDURES

For the realization of this investigation special permits were requested for access to wildlife according to Venezuela wildlife law, in this case the scientific hunting permit was requested, for this a form of Popular Power Ministry for Ecosocialism (MINEC) was completed, as well as the stipulated requirements, attached with the preliminary draft and relevent papers were transferred for one of the investigators to the only ticket office of minec headquearters in Caracas, where the processing of the permit began, said permission was approved allowing the realization of this investigation (anex 2).

The taking samples to the specimens that make up the sample was organized, performing the transfer for specimens sampling that in 2019 were released, once in the installations 2 wood table were set frontally to the water tanks in a flat land terrain, in one wood table was the zoofarm team conformed by the biologists Omar Hernández, Álvaro Velasco Barbieri y Rosangela Blohm, the mesauring tapes and a laptop were placed on this table, and the scale hanging type was hung in a beam of installation, to side of said table at a distance of 1 meter the other wood table was placed for taking blood sampling, in this table was the veterinary doctors Leonel Ovalles, Kevin Garcia y Estefania Acevedo, in said table all instruments necessary for blood draw were placed, as well as the previously backwash and draining syringes with sodium heparin was realized.

It proceeded to perform the specimens handling, contributing to own zoofarm handling, according to exposed activities like morphometry and review previously to reléase. The handling tecnic to use is the same that the zoofarm tecnic personal performs, which consists in manual physical renstraint, capturing the caimans with both hands in water tanks and placing them an elastic band in the snout. Immediately the animals were ubicated in plastic container cages for later be transferred to a place that was to 5 meters approximately where the tables were positioned and where the caimans were taken out individually from the containers for to carry out clinical inspection, later were placed on a table where the measuring tapes were glued, the total length and tail length measures were taken, then the weight was taken with a digital scale hanging type, the caimans were thoracic hanging with a cord to back level, the data colleted were introduced in a digital excel spreadsheet, the caimans were transferred to another table, where the sampling was done, firstly through the manual physical restriction the caimán was placed in sternal decubitus and with a cotton swab impregnated with alcohol the área post occipital was cleaned, the puncture was done in the post occipital venous sinus, the syringe previously backwashed with sodium heparin with its needle was introduced with the bezel facing up at a 45 degree angle, once ubicated the venous sinus was extracted a blood proportion smaller than 1% caimán weight, a 0,5 % of blood volume approximately, the needle was removed pressing the puncture site with swabs, the samples were placed in syringe wrap which were identified with a black acrylic marker, placing a correlated number with the animal plate number, later the animal plate number was searched and was placed on a physical worksheet along with the number to correlate, likewise body condition was evaluated and was placed too in worksheet. At the end, the animals already precessed were placed in plastic container cages of handling for their observation, later they were taken to their water tanks where kept under observation previously to reléase, to guarantee do not show no adverse effect that can limit their development in wildlife.

After the extraction of blood samples, two smears of each sample were perfomed placing a blood drop on an glass microscope slide and spreading it with a coverslip slide and indentifying with a red wax crayón each slide placing the correlative number that make reference to sampled animal number. The smears were fixed with FIXCELL cells fixative placing enough substance for cover the blood spread and letting dry to environmental temperature of fast shape. The slides were placed in a microscope slide boxeo of special design prevent samples for deteriorating, later the samples were transferred to my loyal friend consulting room in Barinas state.

Each record is accompanied with measures and any clinical aspect that was evidenced in the animal, indicative of pathologies or physical abnormalities in addition to phenotypic characteristics.

Once on my loyal friend consulting room laboratory it proceeded to staining of samples with Giemsa colorant wich worked in a proportion 50/50 % and was previously prepared in a test tube in proportion of 3:3 deshionized wáter and giemsa droplets. The solution was placed over the smears already fixed, covering the entire spread and letting the samples stain for 10 minutes. Then the dye excess was rinsed off with distilled wáter without contact and let it dry. Each sample was seen in an optical microscope with the 40 X y 100 X objectives, placing on immersion oil in the smears Surface for can perform the observation with 100 X objetive, to follow to presents hemoparasites detection and identification according to their morphological characteristics, such as shape, size and location.

For one second review and samples check, 50 samples were randomly selected, of wich 25 were undyed for be the second specimen smears, this samples were transferred in a microscope slide boxe of 50 units to Lara state, especially to UCLA parasitology laboratory, where the 25 samples undyed were dyed with Hemacolor by the doctor Maria Forlano, parasitology teacher from UCLA, the samples were inspected widely on an optical microcope with the 40X y 100X objectives. To said samples a third review was carry out with Jorge Arenas, bioanalyst of Bararida zoological and botanical park with more of 26 years of experience, that review was carry out in the installations of Bararida park laboratory, where the samples were observed in an optical microscope.

DATA ANALYSIS AND PROCESSING

The data obtained from data collection, from the sample studied were processed with the statistical softwares: STATISTIX, view. 8.0 y SPSS view. 19.0 through the following techniques.

1. Frequency distributions, percentages, frequency histograms and polygons from positive and negative cases of hemoparasites.
2. Point parametric estimation of the positive cases to hemoparasites proportions, as well as too the weight, body condition and length averages.
3. Test of χ2 of Independence and contingency for relate weight, body condition and length variables with the hemoparasites presence.

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