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CRUORIFILARIA TUBEROCAUDA EBERHARD, MORALES & ORIHEL, 1976 (SPIRURIDA: FILARIOIDEA) IN CAPYBARAS (*HYDROCHOERUS HYDROCHAERIS* LINNAEUS, 1766) FROM VENEZUELAN LLANOS

CRUORIFILARIA TUBEROCAUDA EBERHARD, MORALES & ORIHEL, 1976 (SPIRURIDA: FILARIOIDEA) EN CAPIBARAS (*HYDROCHOERUS HYDROCHAERIS* LINNAEUS, 1766) DE LOS LLANOS DE VENEZUELA

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ABSTRACT

The occurrence of a previously known parasite of capybara in Venezuela a new type of lesion in the kidneys host produced by the presence of filarioid worms of the species *Cruorifilaria tuberocauda* Eberhard, Morales & Orihel, 1976 (Spirurida, Filarioidea) are described, including and evaluation if the burden of filaroids affect the weight of the kidneys and the size of capybaras. A total of 200 filaroid nematodes identified as *C. tuberocauda* parasitizing kidney blood vessels were obtained in 13 of 41 capybaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) (Rodentia, Hydrochaeridae) sacrificed during the 2014 annual harvest in Alto Apure, Venezuela. Cortical cysts of varying sizes, between 0.5 cm and 2.5 cm in diameter, were found in each of these animals. The overall prevalence in the present study was 31.71%, with a mean intensity of 4.88 parasites per host and mean abundance values of 15.38 parasites per host. The coefficient of determination R^2 for each sex attributes to the ratio of foot length and parasitic burden a value of 0.03 to 19.27%, and to the ratio of kidney weight and parasite burden a value of 0.16 to 14.42%, suggesting that the relationship between these variables is weak. The analysis of variance determined that these variables are not significantly different (P = 0.46). No significant relationship was found by sex between growth (foot length) or kidney weight and intensity of worm infections.

Keywords: Capybara – Cruorifilaria – Filarias – Kidney – Size

RESUMEN

Se describe la presencia de un parásito previamente conocido en capibaras en Venezuela, un nuevo tipo de lesión en los riñones del hospedador producido por la presencia del nematodo filarioideo *Cruorifilaria tuberocauda* Eberhard, Morales y Orihel, 1976 (Spirurida, Filarioidea) y se evalúa si la carga de estos afecta el peso de los riñones y el tamaño de los capibaras. Se obtuvo un total de 200 nematodos filaroideos identificados como *Cruofilaria tuberocauda* Eberhard, Morales y Orihel, 1976 (Spirurida, Filarioidea) parasitando los vasos sanguíneos de riñón en 13 de 41 chigüires (*Hydrochoerus hydrochaeris* Linnaeus, 1766) (Rodentia, Hydrochaeridae) sacrificados durante la cosecha anual 2014 en el Alto Apure, Venezuela. Se hallaron quistes corticales de tamaño variables, entre 0,5 cm y 2,5 cm de diámetro en cada uno de estos animales. La Prevalencia general en el presente estudio fue 31,71%, con una densidad absoluta de 4,88 parásitos por hospedador y valores de densidad relativa de 15,38 parásitos por hospedador. El coeficiente de determinación R^2 para cada sexo atribuye a la relación longitud del pie y carga parasitaria un valor de 0,03 a 19,27% y a la relación peso del riñón y carga parasitaria un valor de 0,16 a 14,42% por lo que se considera que la relación entre estas variables es débil. El análisis de varianza determinó que estas variables no son significativamente diferentes (*P = 0,46*). No se encontró relación significativa entre el sexo y el crecimiento (longitud del pie) o el peso y la intensidad de la infección parasitaria.

Palabras claves: Capibara – Cruorifilaria – Filarias – Riñón – Tamaño

INTRODUCTION

Among the species of wildlife in Venezuela, capybara (*Hydrochaerus hydrochaeris* Linnaeus, 1766) has been exploited since the colony (Humboldt, 1826) and is the main alternative source of animal protein for human consumption in several states of the country, on the other hand the use of their skin is restricted to the residents in the rural area of Venezuela. Commercial use of the species begins in the late 1950s (Fergusson, 1990). Between 1962 and 1967 a general prohibition for the use of the wild fauna in Venezuela was decreed. It was during the closure that Ojasti (1973) carried out the studies that allowed knowing the biological information necessary for the structuring and proposal of a management plan according to the characteristics of the resource, which has been adapted and modified over time (González Jiménez, 1995). At present, the program of commercial exploitation of the species in Venezuela, is based on biological information and a management plan that specifies that the extraction is allowed up to a maximum of 20% of the total population of individuals, with a higher live weight to 35 kg and can only be carried out on privately owned lands in the Apure, Barinas, Cojedes and Portuguesa states (Fig. 1).

El Hato El Cedral, located in Apure state, is incorporated into the program of Sustainable Management of natural populations of capybaras in 1991, since 1996 is the largest producer in Venezuela. Population censuses have allowed the allocation of annual harvest quotas of just less than 1.000 to 4.000. The years 1995 and 1997, as well as the period 2008 - 2012 did not realize the annual harvest; the activity is restarted in 2013 with a quota of 3,000 individuals for the seasons 2013 - 2014.

On the other hand, Cañizales & Guerrero (2013) indicated at least 80 different species of parasites (ecto and endo) that have been described for capybaras throughout its natural distribution range. Ojasti (1973, 2011) in his studies on capybara in Venezuela, refers to the presence of a type of endoparasite helminth affecting the lung and kidney in 55.4% of 139 adult animals, while Rodríguez *et al.* (1975) in 38% of 16 adult animals in Colombia. Eberhard *et al.* (1976) taxonomically classified these filarial-type nematodes as *Cruorifilaria tuberocauda* gen. et sp. n (Nematoda:

Filarioidea). This species has been reported in Colombia (Eberhard *et al.*, 1976), Venezuela (Campos-Aasen & Planas, 1986) and Brazil (Costa & Catto, 1994, Nascimento *et al.*, 2000). Microfilariae circulate in the peripheral blood vascular system been found in the skin of top of the head, back an ears (Eberhard *et al.*, 1976, Yates & Jorgenson, 1983) demonstrating peak concentrations between 600 to 1000 hours (Yates & Hellner, 1989). These finding suggest that natural vector may display biting activity on these skin areas and in the morning hours.

Ojasti (1973) observed injuries in the kidneys of capybaras, produced by filarias. *C. tuberocauda* produces a severe arterial injury at the level of kidney and lung, which could generate the formation of arterial thrombosis and areas of degeneration and ischemia. The presence of adult parasites in the blood vessels of the kidney causes an inflammatory response with thickening of the inner walls characterized by the growth of hairy projections towards the lumen as in an attempt to keep the parasite away from the walls (Planas & Campo-Aasen, 1978, Morales *et al.*, 1978). This type of alterations cause chronic renal failure, although it has not documented in capybaras, it is well known in domestic animals as well in laboratory rodents, this disorder specifically affects the growth plate, located at the ends of the long bones, which is formed by cartilage and adjacent bone. Causing variations in the secretion of growth hormone, largely explained by the reduced food intake and nutritional deficiencies characteristically accompanying chronic renal disease and non-specific "per se" of this pathology (Ettinger, 2007). Also, Ojasti (2011) points to foot length as an important indicator of growth in capybaras. We could then assume that the presence of *C. tuberocauda* has an effect on body dimensions in both male and female *H. hydrochaeris*.

The objectives proposed in this study were: to identify the macroscopic findings present in the kidneys parasitized by *C. tuberocauda*, to evaluate the prevalence, absolute density, relative density and infection index of *C. tuberocauda* parasitic populations, and to determine the potential association between the foot length and the kidney weight of the hosts with the parasite burden of *C. tuberocauda*.

MATERIALS AND METHODS

The work area known as the Alto Apure, is characterized by being flooded savannahs during the rainy season. Its physiognomy is mostly open grassland with almost continuous grassland coverage, the canyons and larger rivers are flanked by gallery forests. The main climatic characteristic of this region is the alternation of dry or summer seasons (November to April) and rainy season or winter (May to October), with annual rainfall close to 1.500 mm. The annual average temperature is 27 ° C, and the daily variation is between ± 9.5 ° C. The warmest month is April with an average temperature of 29 ° C.

All samples collected come from adult animals (n = 41) during the beginning of the annual harvest season (March 2014) on Hato El Cedral grounds (7º 23 'N, 69º 20' W) administered by the Instituto Nacional de Tierras with an area of 53,000 hectares, which is located to the west of Apure state, between the towns of Mantecal and Elorza (Fig. 1). This ranch has a system of dikes that diminishes the effect of the seasons when retaining water during the summer and to diminish the extension of the flood in the winter.

The killing takes place in the open air. Once an animal has died, evisceration is performed. Simultaneously, the kidneys were separated by cutting blood vessels and the perirenal capsule, and the foot length (right hind limb) was obtained by measuring from the tip of the middle finger to the base of the heel using a 1:100 scale metal ruler adjusted to one millimeter. The organs were placed in plastic bags and were closed and labeled with sex and foot length. This material was transferred to the field laboratory for initial processing. First, the kidneys were washed in running water to remove unwanted tissue and traces of blood and a first visual inspection was performed to determine some distinctive feature present in the organ. Individual weighing of each kidney was performed using a manual scale weighing 1 milligram, and stored in a clean plastic bag with 4% formalin solution. At a later stage, the organs were macroscopically reviewed in the laboratory, both externally and internally, to record their status and perform the corresponding dissections to collect the filarias. All adult parasites collected were

washed in isotonic saline solution and fixed in 70° Ethanol later. For identification, the criteria of Eberhard *et al*. (1976) were followed. All measurements are given in micrometers (µm), to measurements and figures a Microscope Nikon Labophot YF-21E with Camera DS-Fi1 and Control Unit DS-L2 with software to measurements, an ocular micrometric to verify measurements was used. All parasites were deposited in the parasitology collection of the Museum of Biology of the Central University of Venezuela.

Analysis of data

For the determination of the parasite indices of Prevalence, Mean Intensity, Mean Abundance (Morales & Pino, 1991; Bush *et al.,* 1997) and Infection Index (Guerrero, 2007) were used. The Infection Index is known as number of individuals of a particular parasite species in a single infected host. It estimates the presence of rare but very abundant species and species very frequent but little abundant. For the calculation, the number of individuals of a parasite species present in the total number of both infected and non-infected hosts multiplied by the number of infected hosts divided by 100.

In order to know if there is a relationship between the weight of both kidneys and foot length with parasite burden, simple linear regression and an analysis of variance (ANOVA) were used to determine if there were significant differences in these biometric variables between the sexes.

RESULTS

Although the objective of assessing compliance with the criteria for the selection of animals for commercial use was not formulated, it was possible to verify on the spot (during the harvests 2012, 2013 and 2014) that the selection criteria for adult or old animals were not applied.

A total of 81 kidneys were obtained from 41 individuals sacrificed, one of the animals presented unilateral kidney atresia. The parasitized kidneys externally had no macroscopically visible damage or alteration in size, shape or coloration. By touch, the kidneys had typical consistency. The most important macroscopic findings were observed internally in the sagittal sections of the kidney, (1) thickening and calcification of the walls of blood vessels in the same way as described by Morales *et al*. (1978), Planas & Campo-Aasen (1978), Matos Vieira *et al*. (2006), and Silva *et al*. (2015). For more detailed information of anatomic pathology see the above-mentioned authors. (2) Presence of corticomedullary cysts of sizes between 0.5 cm and 2.5 cm in diameter closely attached to the renal parenchyma with translucent serous fluid delimited by a thin wall (≤0.1 mm) of whitish fibrous tissue. There were no changes in color in the surrounding tissue. All cysts were located between the anterior pole and the middle third of the affected kidneys. No parasitic forms were observed in any of the cysts. These structures have not been previously reported in capybaras. Therefore this constitutes the first known report (Fig. 2).

A total of 200 nematodes were found parasitizing the blood vessels of the kidneys of 13 animals. Females with 150 nematodes were the ones with the highest parasite load against 50 in males. Although the infection was bilateral, the greatest number of parasites (154) was found in the left kidney, the remaining 46 were located in the right kidney. In relation to the number of filarioids found per kidney these ranged from 3 to 64 parasites.

These nematodes were identified as *C. tuberocauda* following the criteria defined by Eberhard *et al*. (1976) as follows: males have two spicules, a large spicule of between 290.00 and 315.00 µm (306.66 ± 14.43) and a small one of between 50.00 and 60.00 µm (55.00 ± 5.00). Total length between 17.72 and 19.02 mm (18.15 ± 0.75), esophageal length between 1.43 and 1.56 mm (1.47 ± 0.07), and a tail length between 145.00 and 155.00 µm (150.00 ± 5.00), females generally have a total length of between 27.50 and 31.00 mm (28.91 ± 1.62), the length of the esophagus between 1.55 and 1.79 mm (1.67 ± 0.16) and a tail length of between 200.00 and 230.00 µm (218.75 ± 14.36) (Fig. 3).

Values of prevalence (%), mean intensity, mean abundance, and infection index by sex are presented in Table 2.

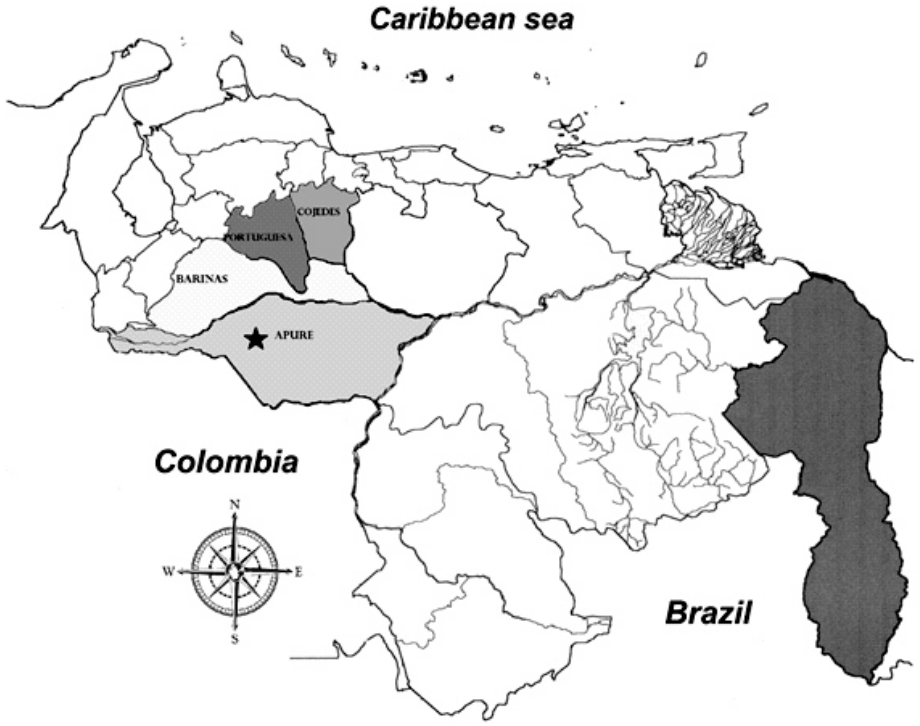

Figure 1. Implementation area of the Capybara Commercial Use Program in Venezuela. ★= Ranch location.

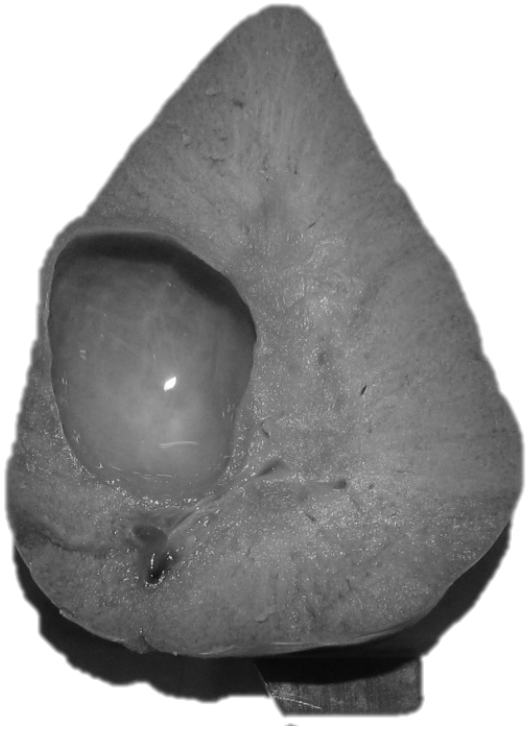

Figure 2. Kidney cross section. The location in the renal cortex is observed. Photo: I. Cañizales. 12X

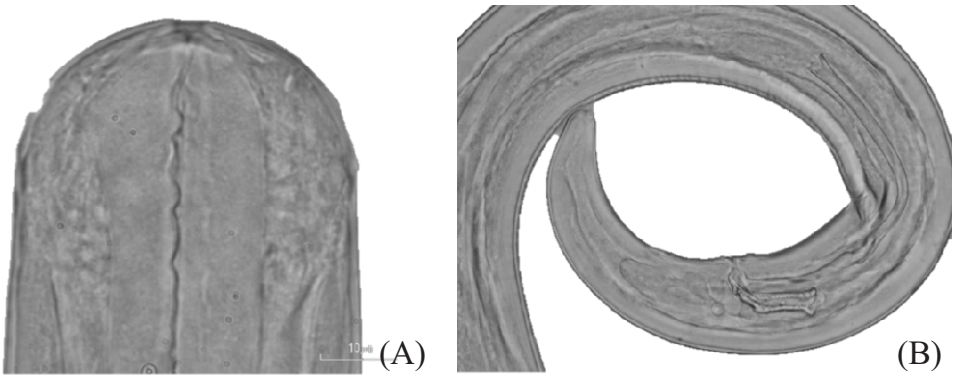

Figure 3. *Cruorifilaria tuberocauda* (A) anterior end (B) tail of the male. Photos: R. Guerrero. 1000X

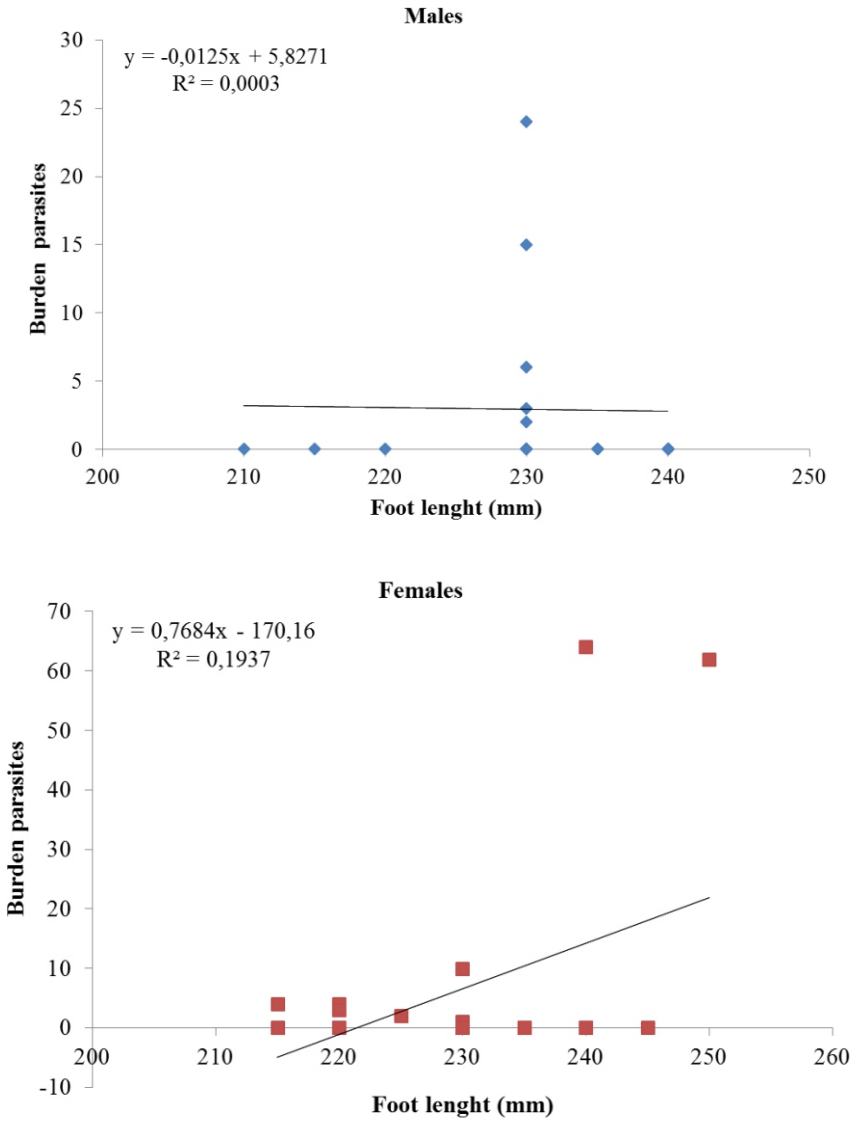

Figure 4. Dispersion diagram of parasite burden and foot length of the hosts by sex.

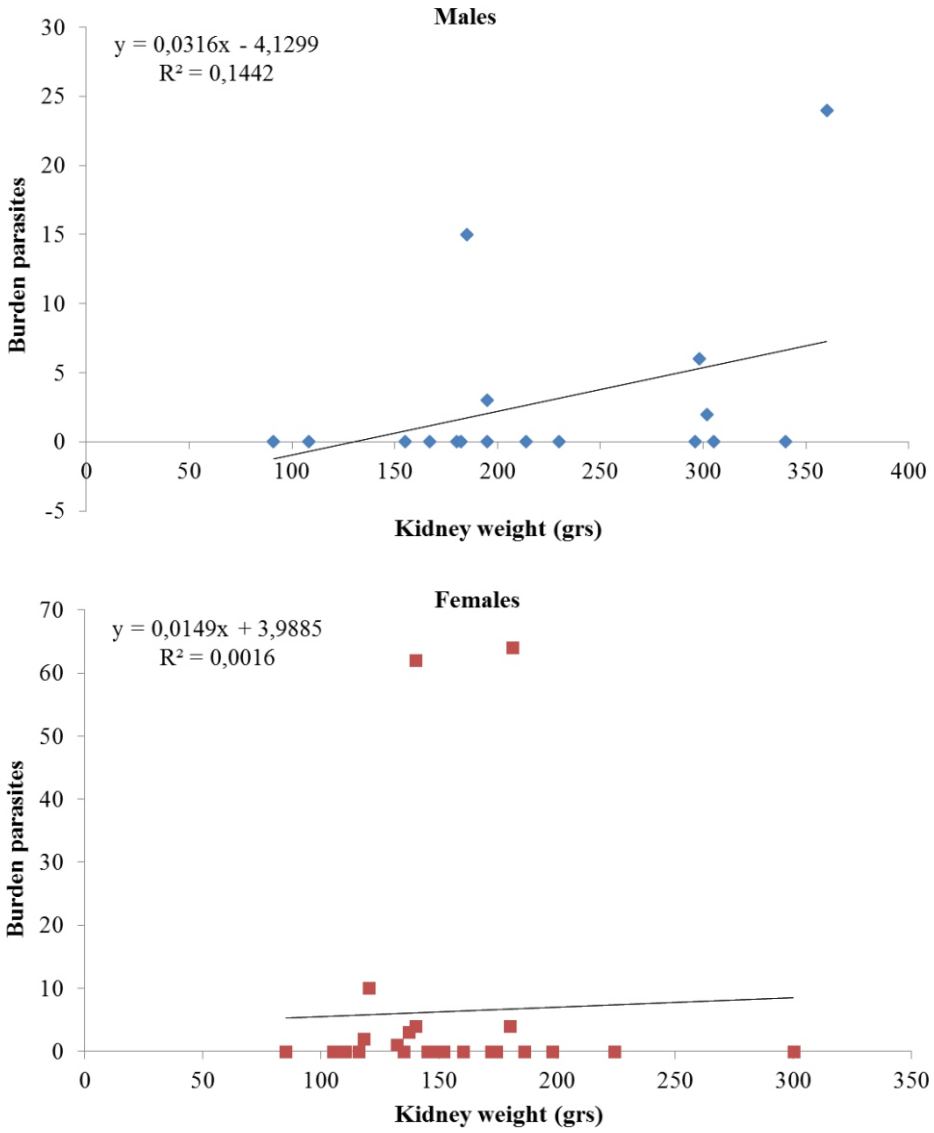

Figure 5. Dispersion diagram of parasite burden and kidney weight of hosts by sex.

Table 1. Biometric data of kidneys and foot length of Capybara.

	Weight (g)			Size(mm)
	Left kidney (Mean ± SD)	Right kidney (Mean ± SD)	Both (Mean ± SD)	Foot length (Mean ± DE)
This study (n = 41)	94,71 ± 37,33	89,90 ± 39,41	181,54 ± 71,33	229,88 ± 9,39
Ojasti, 1973 (n = 110)			188,58 ± 52,95	233,08 ± 7,85

SD = Standar Deviation, n = sample size

Sexual dimorphism in capybaras manifests in males of larger size compared to females, therefore, one could expect variation in parasite burden with respect to sex host in response to this characteristic. Regressions between the foot length and kidney weight of the hosts were made for both sexes. Figures 4 and 5 show the distribution of the data and its trend line. The corresponding R^2 determination coefficients are reported for each sex.

Observing the scatter plots corresponding to males and females, the points show a random type arrangement. However, when graphing trend lines, in the case of males has a negative slope. Therefore, if there is a relationship between the variables, it will not be linear. In females the trend line has a positive upward slope. This suggests that there is some relationship between the variables, although clearly not linear. In relation to the values reported by each determination coefficient for each sex, these vary from 0.0003 to 0.1937, that is 0.03 to 19.27% of the variation in the parasitic burden can be attributed to relationship with the length of the foot, so it is considered that the relationship between these variables is weak.

Scatter plots corresponding to males and females, the points show a random type arrangement. However, when plotting trend lines, in both cases they have a positive upward slope. This suggests that there is some relationship between the variables, although clearly not linear. In relation to the values reported by each determination coefficient in each sex, these vary from 0.0016 to 0.1442, that is 0.16 to 14.42% of the variation in the parasitic burden can be attributed to relation with the weight of the kidney, reason why is considered that the relation between these variables is weak.

To evaluate whether or not differences between sexes for foot length and kidney weight with parasite burden were significant, an analysis of variance (ANOVA) was performed. The analysis determined that these variables were not significantly different ($F = 1.03$; $P = 0.46$, $Fc = 1.69$).

Table 2. Prevalence, mean intensity, mean abundance, infection index of *Cruorifilaria tuberocauda*.

	Prevalence%	Mean intensity	Mean abundance	Infection index
Males	29.41 (5/17)	2.94	10.00	0.87
Females	33.33 (8/24)	6.25	18.75	2.08
General	31.71 (13/41)	4.88	15.38	1.55

(/) = animals parasitized/simple size

DISCUSSION

The artificial selection of bigger size animals favoring the survival of smaller or the random selection of animals below the norm established in the area of work and that to a certain extent affects the populations dynamics of capybaras, such as those associated with reproductive processes, could explain the observed differences between the values obtained in kidney weight and foot length by Ojasti (1973) and this study of 3.73% and 1.37 % respectively (Table 1).

Although injuries caused by *C. tuberocauda* have been described in detail by Eberhard *et al.* (1976), Morales *et al.* (1978), Planas & Campo-Aasen (1978), Nascimento *et al.* (2000) and Matos Vieira *et al*. (2006), in none of these studies is pointed the presence of cortical renal cysts associated with the presence of this nematode. Macroscopically, the cysts are mainly located in the renal cortex and vary from 0.5 cm to 2.5 cm in diameter with the presence within of an aqueous and translucent liquid (Fig. 2).

In order to be able to compare our results of total parasite burden with other studies with similar

sample size, age-composition in an area with similar characteristics, we must mention that we did not find published works that complied with these requirements. When we compared the data of this study, 200 parasites in 41 adults' animals, with the findings of Nascimento *et al*. (2000) of 380 parasites in 36 animals in Mato Grosso in Brazil, it could be concluded that the parasitic burden in our case is lower however the influence by the age-composition of the host sample must be considered (25 individuals adults and 11 individuals of six months old). This gives us a mean of 4.88 parasites/host in this study compared to the 10.56 parasites/host study Nascimento *et al*. (2000). The overall prevalence in the present study was 31.71%, lower than the 55.4% reported by Ojasti (1973), 52.7% by Nascimento *et al.* (2000) and 38% by Rodríguez *et al*. (1975). Likewise, the mean intensity values of *C. tuberocauda* in the present study were 4.88 parasites per host and differ from that found by Costa & Catto (1994) in Pantanal chigüires from the state of Mato Grosso do Sul of 8 parasites per host, and Nascimento *et al*. (2000) who observed a mean intensity of 20.70 parasites per host. On the other hand, the mean abundance values of *C. tuberocauda* in the present study were 15.38 parasites per host, exceeding Nascimento *et al*. (2000) of 10.94 parasites per host finding. Likewise, the infection rate with an overall value of 1.55 allows us to conclude that in general the natural populations of capybaras in this study are less parasitized than the natural populations in other countries within the natural range of the species.

Much has been discussed about the effect that sex and host size may have on parasite burden (Folstad & Karter, 1992; Schalk & Forbes, 1997; Krasnov *et.al.*, 2005; Gorrell & Schulte-Hostedde, 2008) in capybaras sexual dimorphism manifests in males of larger size compared to females, so some variation in response might be expected to this characteristic. In this study the results obtained from the corresponding analyzes determined that there is no direct relationship between the variables.

On the other hand, the definitive location of nematodes adult form in circulatory system in a mammalian host is not unique of *C. tuberocauda,* there are other species of the Onchocercidae family that live in arteries such as *Dirofilaria immitis* (Leidy, 1856), *Acanthocheilonema reconditum* (Grassi, 1890), in which a hematophagous vector participates as a transmission mechanism of microfilariae. In a similar way *C. tuberocauda* also is not the only known species whose is present in the kidney of a definitive mammalian host, *Dioctophyma renale* (Goeze, 1782) commonly referred to as the "giant kidney worm" is a parasitic roundworm (Ascaridida: Dioctophymatidae) whose mature form is found in the kidneys of mammals.

Eberhard *et al*. (1976) proposed that this rodent is not the natural host of the parasite due to the severity with which the parasitized tissue of the kidney responds to this species, however, the extent of organ damage by a parasite is not necessarily evidence of an abnormal host. *D. renale* usually destroys the kidney completely and canids, felids and mustelids serve as the usual definitive hosts.

Finally, among the hematophagous arthropods present in the "Hato El Cedral" we can point out acari (Ixodidae, Macronyssidae), lice (Menoponidae) and mosquitoes (Culicidae). Villarroel & Navarro (2014) report the genus *Mansonia*, *Anopheles* and *Culex* which are involved in the transmission of *D. immitis*, *Setaria labiatopapillosa* (Alessandrini, 1838), *Acanthocheilonema reconditum* among others, as well as *Mansonia* and *Coquillettidia* that have been involved in the transmission of different arboviruses such as Venezuelan Equine Encephalitis Epizootic and West Nile Virus, *Aedomyia squamipennis* (Lynch Arribálzaga, 1878) which is a vector of Gamboa virus and avian malaria (Machado-Allison 1982). *Anopheles nuneztovari* (Gabaldón, 1940), the most important vector of human malaria in the Venezuelan plains, especially in Barinas and Apure, would be reasonable to consider that one of the species representing these genera participates as potential vectors of *C. tuberocauda*. However, Lefoulon, Bain, Bourret, Junker, Guerrero, Cañizales *et al*. (2015) in a study of the phylogeny and evolution of filarias, mention *C. tuberocauda* as part of a clade composed by the genera *Dipetalonema*, *Acanthocheilonema*, *Monanema*, *Cercopithifilaria*, *Yatesia* and *Litomosoides* that show adaptation for its transmission to a more appropriate group of vectors: ticks and mites. In

summary, so far the vector responsible for the transmission of *C. tuberocauda* is not known.

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