COMMUNICATION

ON THE IMPORTANCE OF ALPHA BEHAVIOR INTEGRITY IN MALE CAPIBARA Hydrochoerus hydrochaeris (Mammalia: Rodentia: Caviidae) FOLLOWING IMMUNO-CONTRACEPTIVE TREATMENT

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Derek Andrew Rosenfield 1,2 & Cristiane Schilbach Pizzutto 1,2

1,2 Department of Animal Reproduction/Wildlife, Faculty of Veterinary Medicine and Animal Science, University of Sao Paulo, A. Prof. Orlando Marques de Paiva 87, Cid. Universitaria, Sao Paulo, Brazil 05508-270.
1 dro@usp.br (corresponding author), 2 crissp@usp.br

Abstract: As the human population continues to grow, habitat for wildlife shrinks, driving fauna either into extinction or into new habitats, which can create new problems. In Brazil, the Capybara Hydrochoerus hydrochaeris has become a pest by invading urban and agricultural areas. These mammals quickly multiply owing to abundant food supply and a lack of natural predators, and they can serve as amplifying hosts for Rickettsia rickettsii, the pathogen of potentially life-threatening Brazilian spotted fever. Species-specific population management strategies that respect public opinion and consider animal welfare are required for the effective mitigation of this tick-borne zoonotic disease. In order to control Capybara populations it is necessary to take into account their social dynamics, which are centered on polygynous dominant males with hormone-driven secondary sexual characteristics. To be a viable management tool, a contraceptive strategy targeting these males must preserve their social status to prevent other males from replacing them. As part of a larger research project on the efficiency of anti-Gonadotropin-releasing hormone (GnRH) vaccine treatment in free-ranging Capybaras, the aim of this study was to observe the impact of this treatment on alpha male and overall social group behavior. At the end of the 18-month study, there were no recorded births involving the immunized animals, and alpha male sexual characteristics and group integrity were preserved. These results encourage the use of this anti-GnRH vaccine as an alternative population control tool in male Capybara.

Keywords: Agonistic behavior, Brazilian spotted fever, Gonacon, Rickettsia, secondary sexual characteristics, wildlife population control.
INTRODUCTION

*Hydrochoerus hydrochaeris*, commonly known as Capybara, the world’s largest rodent species, endemic to South America, and are habitat generalists surviving in open grasslands and scrub vegetation. Capybaras are semi-aquatic so stay close to water, which is used as their principal getaway and serves as a place for thermoregulation, defecation, mating, as well as an important food source (Mones & Ojasti 1986; Magnusson 1998; Moreira et al. 2012; Elias 2013). Intensive anthropogenic activities have dramatically changed the landscapes and habitats where Capybara live. With diminishing natural space and an increase in agricultural and urban areas, these animals are re-occupying and thriving in their human-modified habitats.

Their social structure is based on polygyny (harem), with one dominant (alpha) male, females that are divided into dominant (breeding females) and subordinate females, male and female juveniles, and, depending on the season, pups. In Brazil, a wild herd can reach up to 50 members (Macdonald 1981). Isolated Capybara, known as satellites, can often be seen maintaining a certain distance from the main group; these are sexually mature males forced out by the alpha male (Image 1).

Subordinate females, although sexually mature, do not mate with the alpha male; their restraint is due to interactions with dominant females and their social stimuli, which is believed to cause reproductive suppression, either physically, endocrinologically, or by olfactory cues (Maldonado-Chaparro & Blumstein 2008). However, some subordinate females have been observed leaving their group for short periods of time to seek out nearby satellite males to mate with, as observed during this present study.

Due to several contributing factors, such as the loss of natural predators, the Capybara’s ability to quickly adjust to agricultural and urban settings, their tolerance to human presence, the abundance of available foods, combined with their high proliferation rates, have allowed the Capybara to become Brazil’s second most important pest-species, the other being Wild Boar (Pedrosa et al. 2015). Under these conditions Capybaras attain large population sizes, with herd numbers that can reach over 100 individuals which creates traffic accidents, damage to private property, invasion of public and private spaces, and destruction to crops, particularly corn and sugarcane plantations (Ferraz et al. 2003; Labruna et al. 2007; Labruna 2013; Felix et al. 2014; de Oliveira Vieira et al. 2015; Abreu Bovo et al. 2016).

The main concern, however, is the threat to human health as Capybara are associated with the maintenance and spread of the tick-borne disease Brazilian spotted fever (Portuguese: febre maculosa). Capybara are considered an amplifying host for this emerging vector-borne zoonosis caused by the potentially deadly bacterium *Rickettsia rickettsii*, which is spread by ticks of the genera *Amblyomma* sp. (Fortes et al. 2011; Labruna, 2013; Brites-Neto et al. 2015).

They fulfill five requirements to be considered a good amplifying vertebrate host for *R. rickettsii*:

1. be abundant in the endemic area, (2) be a good host for the ticks, (3) be susceptible to Rickettsia infection, (4) have high population growth rates, and (5) have enough bacteremia counts to infect ticks (Labruna et al. 2009).

Although there are other native wildlife species reported to host *R. rickettsii*, such as dog, horse, opossum, among others (Labruna et al. 2009; Milagres et al. 2010), there are a number of reasons that Capybara are the major contributing factor for *R. rickettsii* infection in endemic areas. They exclusively occupy areas close to bodies of water and move slowly, making them conducive for ticks to infest in large numbers and feed upon. Some tick species such as *Amblyomma dubitatum* are highly specific to Capybaras and rarely feed on other host species. Humans, however, may become accidental hosts (Guglielmone et al. 2006; Labruna et al. 2007; Beati et al. 2013; Brites-Neto et al. 2015).

Several field studies and stochastic models have been developed that have reported spotted fever transmission dynamics, postulating that birth-rate reduction not just can directly control Capybara population growth, but potentially slow disease transmission (Sonenshine & Mather 1994; Labruna et al. 2002; Federico & Canziani 2005; Polo et al. 2017; Polo et al. 2018; Rosenfield et al. 2019).

In an effort to control these fast-growing super-populations, several research projects are being conducted, seeking methods that are effective in managing populations while conforming to environmental protection laws and public opinion. Capybara, as it’s categorized as Brazilian native fauna, is protected from hunting, slaughter, and abuse (Presidência da República 1981; Rodrigues 2013).

In Brazil, Capybara potentially reproduces all year round, however, they are constrained by environmental factors, food availability and human impacts. As the principal breeder, the alpha male protects the herd and mates with many females. Focusing on the sterilization of the dominant male could be a population control strategy of choice, provided that the procedure does
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not alter its dominant status (Alho & Rondon 1987; Rodrigues 2008; Paula & Walker 2013). Capybara are fiercely territorial, protecting harem and habitat, driving out potential male intruders or subordinate males that attempt to challenge the alpha male (Herrera & Macdonald 1993); thus, the importance to maintain testosterone production which influences their secondary sexual characteristics and dominance (agonistic) behavior. For this reason, vasectomy was considered initially a suitable intervention as sperm conduction is interrupted, yet, leaving the gonad function intact so a continuation of steroidogenesis is ensured (Meira et al. 2013). If performed correctly, it is completely effective. On the downside, the logistics, cost, skill availability and access to the testes which is in an intra-abdominal position, are more challenging. The biggest dilemma, despite being considered a minimally invasive surgical procedure, is the time for recovery. Capybara, when injured, sick, or during labor, distance themselves from the group until healing is complete (D.A. Rosenfield xi.2016 – xii.2018). Observations indicate that vasectomized males distance themselves from the group for up to 10 days, potentially allowing competitors to move in and take over, jeopardizing the efforts to manage the population growth.

Additionally, subordinate males are known to breed opportunistically, even as much as 40% of the overall growth rate (Rodrigues 2008), which is initiated by subordinate females temporarily leaving the main group (Labruna Marcelo & Fernanda Nunes pers. comm. 20.ix.2018). In this case, an alternative method to consider is tubal ligation (tubectomy) in all sexually mature females. The concept is analogous to the deferentectomy procedure in males, with the intent to inhibit gamete transmission but preserve gonadal steroidogenesis, and, hence, social behavior/group stability.

In general, we can organize the breeding hierarchy into one alpha male and several dominant females as the principal breeders. Subordinate females (believed to be in reproductive suppression due to the presence of dominant females), and/or their opportunistic mating with external (satellite) males (Fig. 1), which postulates three distinct population control strategies:

Contraceptive strategies

1) The immuno-contraceptive treated alpha male effectively maintains agonistic conduct and secondary sexual characteristics. They successfully defend against potential intruders; however, the alpha male does not
mate with dominant females. Subordinate females opportunistically and temporarily leave the group to join nearby satellite males to mate (Fig. 2). After the mating event, the now pregnant female returns to the group and remains there during gestation. Following birth, the pups are brought up in an allo-parental manner, as commonly observed in Capybaras (Nogueira et al. 2000).

2) Due to the castration, the original alpha male loses agonistic conduct and secondary sexual characteristics. Growing males, sexually mature, or dominant growing satellite males challenge the alpha male, leading to his defeat and consequently driving the ex-alpha male out of the group or even killing him (Fig. 3). The new (untreated) dominant male will become the alpha male and restart the mating process.

3) The treated alpha male does not leave the group post-treatment and maintains alpha associated conduct and secondary sexual characteristics. Alpha male is now infertile (Fig. 4), but the group’s social structure is stable. Also, treating all satellite males and all sexually mature females will prevent opportunistic mating encounters with satellite males.

Illustrated contraceptive strategies

In order to find alternative contraceptive methods that would address the weaknesses of currently employed population control strategies in Capybaras, an intensive literature review on contraceptive methods in wildlife was conducted. The objective was to match most of the desired characteristics of a contraceptive agent, which would include antifertility effectiveness of more than 90%; long-term effect of more than 12 months; with very little to no adverse effects (physiological/behavioral). Especially, considering a polygynous society, like Capybara, the importance of maintaining the dominant male’s agonistic behavior is supreme. Furthermore, it is applicable in both the sexes; represents no risks to pregnant females; potentially reversible; easy and safe application; allows for remote drug delivery (long-distance darting); does not provoke environmental pollution; does not have contraceptive effects when entering the food-chain, and lastly, is economically viable and available (Figs. 2–4).

The anti-GnRH vaccine GonaCon™ was selected as it conformed to most of these conditions (Asa 2005; Gionfriddo et al. 2008; Ajadi & Oyeyemi 2015; Rosenfield 2016). The objective of this research was to demonstrate the effectiveness of an anti-GnRH immuno-contraceptive (Gonacon™) in reducing population growth in the capybara without interfering with the behavioral characteristics of the alpha male.

MATERIALS & METHODS

Location

A large man-made water pool (Olympic Lake, Portuguese: Raia Olimpica) surrounded by diverse
vegetation and extended grassy areas, used for the university’s aquatic sports activities, was selected in Sao Paulo (Image 3). The environmental conditions are very similar to the natural Capybara habitats, allowing for a unique opportunity to observe free-ranging Capybaras in an open confined setting.

**Identifying males**

At first sight, the urogenital apparatus is not easily distinguishable between male and female Capybara, as the male penis is situated within a large anogenital invagination. In sub-adult males, gender can be confirmed by palpation and exposure. Capybara alpha males have specialized androgen-driven secondary sexual characteristics (SSC), such as a prominently developed nasal and perianal glands for scent-marking (Image 2a,b). The testes, in immature males, are located subcutaneously in the inguinal region, whereas in dominant males, they migrate from the inguinal region towards the area of the inner/upper thigh, becoming slightly visible (Image 2c).

**Veterinary intervention**

Two groups were selected for the study and were based on their population size and pest status: Group 1 consisted of more than 40 individuals and Group II of seven individuals. The socio-sexual and reproductive behavior of male and female Capybaras were recorded for approximately 100 hours pre- and >120 hours post-treatment using the continuous focal observation method (Martin & Bateson 2007) between June 2016 and December 2018.

In Group I, three individuals (male n=1, female n=2), and in group II, six individuals (male n=2, female n=4) were treated with the anti-GnRH vaccine GonaCon™. The vaccine was administered intramuscularly in the larger muscle group of the hind leg. The rest of the population served as control.

This project-specific ethogram (Table 1) was used to assist in identifying any treatment-associated alterations, allowing an interpretation of cues of a successful antifertility method and the integrity of the alpha male’s agonistic behavior. This is essential for maintaining the group’s social stability and preventing an intruder from mating, and hence, providing an appropriate population growth management tool.

**Contraceptive effect analysis**

As part of the evaluation process of the anti-fertility effect, steroid-hormone, spermogram, biometry, and testicular morphology were employed. At the end of the study period, males were hemi-castrated for further histological investigation of the testicular parenchyma. These specific findings are submitted for publishing elsewhere.

**RESULTS**

**Effects of treatment**

The immunized alpha males showed oligospermia, compared to control, while their agonistic behavior and secondary sexual characteristics were preserved (Tables 1 & 2).

**DISCUSSION**

The leadership dynamics observed of all involved males was very compelling, as it proved that the immuno-contraception was effective in rendering the treated males infertile, while concurrently, preserved their alpha male behavior, and thereby the group’s integrity. Confirmed by the behavioral observations made of an untreated sexual mature male, used as the control variable, demonstrated during involved transitional dynamics the take-over of a leader-less group as the new alpha male, consequently producing offspring.

Positive antifertility effects of the immuno-contraceptive was confirmed in similar studies and in various species; however, where the findings differ are the observations that state the loss of agonistic behaviors in males (Snape at al. 2011; Donovan et al. 2013; Doughty et al. 2014) while others report no significant changes (Massei et al. 2008; Young, 2018), including the present work.

Also important is the fact that the treated animals did not distance themselves from the group for recovery, hence, preventing any opportunity for a satellite male
### Table 1. Experimental interventions and outcomes. (PDB: preserved dominant behavior; SSC: secondary sexual characteristics).

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Target</th>
<th>Intervention</th>
<th>Result</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alpha male</td>
<td>Immunised</td>
<td>Oligospermia</td>
<td>PDB (driving out any male invader)</td>
</tr>
<tr>
<td>2</td>
<td>Alpha male</td>
<td>Immunised</td>
<td>Oligospermia</td>
<td>PDB</td>
</tr>
<tr>
<td>3</td>
<td>satellite male control male</td>
<td>No intervention</td>
<td>Produced offspring</td>
<td>Males mated with females from group 1 and 2, that left the group temporarily. In the absence of a dominant male, satellite males will take over the group.</td>
</tr>
</tbody>
</table>

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**Figure 2.** Contraceptive strategy I. Treated dominant male, infertile but with preserved alpha characteristics/behavior. No mating between treated dominant male and dominant females (red arrow). Opportunistic mating between satellite male and subordinate females (may leave the group temporarily). © D. Rosenfield, 2019

**Figure 3.** Contraceptive strategy II. Effects of castration: Loss of male’s alpha characteristics (mainly agonistic behavior), subordinate/satellite males challenge the alpha male for dominance, eventually taking over the group. Effects of vasectomy: Alpha male will distant himself from the group for a short period of time (recovery phase from surgical injury), leaving the group temporarily without alpha male. Opportunistic window for a satellite male to invade/take over the group. © D. Rosenfield, 2019
Table 2. Ethogram of recorded behavior.

<table>
<thead>
<tr>
<th>Ethogram Alpha Male</th>
<th>Observations Control Alpha Male</th>
<th>Treated Alpha Male</th>
<th>Satellite Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any visual treatment-related discomfort</td>
<td>Separating from the group immediately after treatment; Apathetic; no foraging; allowing an intruder to get close to the group/females</td>
<td>n/a</td>
<td>The treated alpha did not distance himself at any moment post-treatment. All alpha related conducts remained intact.</td>
</tr>
<tr>
<td>Vigilance</td>
<td>Alpha male remained at a certain distance in a sitting or ventral decubitus position watching over the group</td>
<td>Confirmed</td>
<td>Confirmed</td>
</tr>
<tr>
<td>Relocation</td>
<td>Leading group to a different location (for better foraging grounds, or for safety)</td>
<td>Confirmed</td>
<td>Confirmed</td>
</tr>
<tr>
<td>Scent marking</td>
<td>Marking territory by rubbing with nasal gland surface or perianal gland surface over stationary objects; urinating onto the ground</td>
<td>Confirmed</td>
<td>Confirmed</td>
</tr>
<tr>
<td>Courtship behavior</td>
<td>Seeking physical contact with females. Testing receptivity for mating by sniffing urogenital region, pushing snout into female's flank, or snout. Putting head onto female's dorsal pelvic region.</td>
<td>Confirmed</td>
<td>Frequent contact (sniffing) but no mating conduct</td>
</tr>
<tr>
<td>Mating</td>
<td>Male continuously follows the female. Frequent mounting attempts by placing upper torso onto female's lumbar/pelvic region, performing a thrusting motion with the pelvis.</td>
<td>Confirmed</td>
<td>Not observed. Possible attempts, but infertile male.</td>
</tr>
<tr>
<td>Agonistic (aggressive) behavior</td>
<td>Attacking, fighting, and chasing the intruder</td>
<td>Confirmed</td>
<td>Confirmed</td>
</tr>
</tbody>
</table>

Noteworthy, alpha males, given the right circumstance, would leave their group in order to take over a group with a larger number of females, as observed twice.

There were no significant phenotypic or behavioral alterations, nor any pathological adverse effects in the treated animals. Although the treated alpha males were considered infertile, their secondary sexual characteristics and agonistic behaviors appeared to be preserved, as well as the groups’ overall social integrity, which is an important key fact to successfully managing the population of this species, and exceeded all minimum expectations.

Other currently considered male fertility strategies, such as castration and vasectomy, which are 100%
effective and do have their merits when employed in the right situation, seem less adequate when considering large-scale intervention in the field, considering logistics, as well as animal well-being.

Furthermore, as the findings suggest, injured or sick individuals tend to retreat from the group until recovered, which can take several weeks, representing a window of opportunity for a fertile rival male to take over the group, undoing any population control attempt.

In regard to the relevance to public health, specifically for Brazil and spotted fever, based on a stochastic model (Polo et al. 2017), that indicated a birth-rate reduction of ≥ 90%, the etiological agent of the tick-borne zoonotic disease, hypothetically, could be controlled after two years of intervention, utilizing the alternative population control method for Capybaras described in this work.

The observations conducted over the study period of 18 months suggest that the birth rate reduction needed to directly manage a Capybara population, and indirectly the dynamics involved in maintaining and spreading *R. rickettsii*, could be achieved.

**CONCLUSION**

When it comes to population control, no one solution fits all situations. Each is unique and requires a specific study to choose the most appropriate solution. In free-ranging Capybaras, being able to treat the alpha male, satellite males, as well as all dominant females, seems to be the most promising method for controlling their population growth with the highest success rate. Nevertheless, it is important to understand that all efforts are temporary, and in order to maintain functioning population management, this method must be practiced continuously.

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