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## Effects of surgical and chemical sterilization on the behavior of free-roaming male dogs in Puerto Natales, Chile

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#### ABSTRACT

Population management of free-roaming domestic dogs (Canis lupus familiaris) is of interest due to the threat these animals pose to people, other animals and the environment. Current sterilization procedures for male dogs include surgical and chemical methods. However, little is known about how these procedures affect their behavior. The primary objective of this study was to investigate changes in selected behaviors following chemical and surgical sterilization in a male free-roaming dog (FRD) population in southern Chile. We also examined the association between serum testosterone levels and behaviors thought to be influenced by circulating androgens. A total of 174 dogs were randomly assigned to either a surgical or chemical sterilization group, or a control group. At the onset of the intervention period, 119 dogs remained and 102 dogs successfully completed the study. Each dog was monitored pre- and post-intervention using video recordings, GPS collars, and blood samples for the measurement of testosterone. Analysis of behavior revealed that surgically castrated dogs showed no reduction of sexual activity or aggression when compared to their pre-intervention behavior. Chemically sterilized dogs showed a statistically significant increase in dog-directed aggression, but no change in sexual activity. There was no change in home range size in any groups between the pre- and post-intervention measurement. We found no consistent association between levels of serum testosterone concentration and behavioral changes in any of the groups. This study presents the first detailed behavioral observations following surgical and chemical sterilization in male FRDs. The information generated is highly relevant to communities struggling with the control of FRDs. Complementary studies to further our understanding of the effects of male sterilization on the behavioral and reproductive dynamics of FRD populations are needed.

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#### 1. Introduction

Population control and management of free-roaming domestic dogs (*Canis lupus familiaris*) is gaining in interest because of the threat they pose to public, animal and environmental health and welfare (Butler et al., 2004; Morgan and Palmer, 2007; Otranto et al., 2009; Dalla Villa et al., 2010; McKenzie, 2010; Garde et al., 2013a). In developing countries, where the issue is more prominent (Dalla Villa et al., 2010), sterilization is the principal strategy promoted to halt reproduction and/or to modify undesirable behav-

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iors. However, sterilization efforts are usually focused on female dogs because it is considered a more effective population control approach when resources are limited, and/or where there is cultural opposition to the removal of the testes in male dogs (Levy et al., 2008; McKenzie, 2010). There is increasing public pressure in some countries to include male dogs in sterilization campaigns in the hope that it will reduce reproduction and undesirable traits such as aggression and roaming. This is creating an imminent need to evaluate how different methods of sterilization of male dogs may impact their behavior.

A series of studies published primarily between the 1970s and 1990s suggested that surgical castration of male dogs improved their behavior with regards to inter-male aggression, urine-marking, roaming and mounting (Hart, 1968, 1974, 1979; Le Boeuf,

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1970; Hopkins et al., 1976; Write and Nesselrote, 1987; Knol and Egberink-Alink, 1989; Gershman et al., 1994; Maarschalkerweerd et al., 1997; Neilson et al., 1997) possibly due to the reduction of testosterone (Hart and Eckstein, 1997; Giammanco et al., 2005). Other studies produced contradictory findings including no behavioral changes in surgically castrated dogs, or significant negative post-castration changes such as increased aggression, fearfulness, excitability, anxiety and decreased trainability (Hart, 1968; Salmeri et al., 1991; Hart and Eckstein, 1997; Bennett and Rohlf, 2007; Farhoody and Zink, 2010). An extensive review of the related literature conducted in 2010 (McKenzie, 2010) concluded that the findings do not demonstrate clear behavioral outcomes following surgical castration in male dogs. Moreover, some of these studies suffered from methodological limitations; they tended to be descriptive, subjective (based on owner opinion), non-quantitative, retrospective, based on small sample sizes, and without the use of control groups (McKenzie, 2010). None of these studies were conducted on free-roaming dog (FRD) populations.

While traditional surgical sterilization of male dogs may not be popular or accepted in many countries, chemical sterilants, such as zinc gluconate neutralized with arginine (Esterilsol  $^{\text{TM}}$ , Zinc gluconate 13.1 mg/ml, L-Arginine 34.8 mg/ml, Ark Sciences, 50 South Buckout St. Irvington, New York, 10533), have been gaining in popularity, particularly among organizations and governments conducting massive sterilization campaigns (Levy et al., 2008). The procedure is relatively quick, it is inexpensive, testes are maintained, and sterility is permanent (Oliveira et al., 2012). This is an especially attractive option for some developing countries because of their limited resources to allocate toward dog management and their cultural opposition to the removal of testes (Levy et al., 2008). However, two studies, each using a different formulation of zinc gluconate, have demonstrated a variable effect on circulating levels of testosterone (Oliveira et al., 2012; Vanderstichel et al., 2015). This emphasizes the need for information on the potential behavioral changes that might be expected following chemical sterilization.

Chile is a developing country with large numbers of FRDs (Garde et al., 2013b). Although canine rabies was eliminated from the country in 1972 (Laval and Lepe, 2008), Chile continues to struggle to control a range of other canine-associated problems such as livestock and wildlife depredation, animal welfare, and public health issues including dog bites and attacks, environmental contamination, and canine zoonoses such as *Echinococcus granulosus* (Schenone et al., 1999; Acosta-Jamett, 2010; Silva-Rodriguez et al., 2010). These issues result in the control of FRD populations being a high priority for local municipalities and the national authorities.

If it could be demonstrated that sterilization contributes simultaneously to a reduction in reproduction and undesirable behaviors in male dogs, managers may be more likely to promote the sterilization of both females and males. The primary objective of this study was to investigate changes in behavior following chemical and surgical sterilization in a free-roaming male dog population in southern Chile. We also examined the association between serum testosterone levels and particular behaviors thought to be influenced by circulating androgens. Information about predictable behavioral outcomes following different types of sterilization alternatives is essential in enabling FRD managers, veterinarians and dog owners to make informed decisions.

#### 2. Materials and methods

#### 2.1. Study site, participant enrolment and sample size

Puerto Natales is a city belonging to the Province of Ultima Esperanza, located in the Magallanes Region of Chilean Patagonia. There are 18,505 human inhabitants according to the 2012 national census (http://www.censo.cl/; accessed 09.12.13). In 2009, a canine

census conducted by the Ministry of Agriculture revealed an estimated population of 3,515 dogs in urban areas; 66.2% of the total population were intact males, and a large proportion of owned dogs were free-roaming (Servicio Agrícola y Ganadero, 2010). Prior to participant enrolment, we aimed to include 150 dogs in the study. While mortality rates in very young puppies can be as high as 70-80% (Boitani and Ciucci, 1995; MacDonald and Carr, 1995; Pal et al., 1998), we included a broad age range of healthy, vaccinated subjects. We therefore anticipated our combined mortality and losses to follow-up to be about 40% for all dogs in our study, resulting in a final sample size of 30 or more per group. Based on previous literature, we expected that approximately 40-60% of the surgically castrated dogs would show changes in behavior (Neilson et al., 1997; Maarschalkerweerd et al., 1997; Heidenberger and Unshelm, 1990). Therefore, even with the 40% expected losses due to high mortality rates in free roaming dogs along with loss to follow-up, we anticipated that 12-18 out of 30 dogs would show a change in at least one area of behavior. We visited every house in the selected neighborhoods, and included all dogs that met the requirements of the study.

Participant enrolment took place between May and July 2011, in 8 peri-urban neighborhoods of Puerto Natales chosen by the Ministry of Agriculture and the Municipality of Puerto Natales because of their high populations of FRDs and proximity to rural areas and animals (Fig. 1). All occupied households in the selected neighborhoods were visited, and residents were informed of the study and of the following enrolment criteria: owners 1) regularly allowed their intact male dog access to the street, 2) intended to reside in Puerto Natales for the duration of the study, 3) were willing to have their male dogs randomly assigned to a surgical or chemical sterilization or control group and 4) would allow us to video record (film) their dog pre- and post-sterilization. Consenting owners of male FRDs were enrolled in the study and were asked to sign an informed consent agreement. Dogs were then randomly assigned to intervention groups (chemical, surgical or control) using an online randomizer program (Randomizer, http://www.randomizer.org/ accessed 01.12.12). Field personnel conducting data collection remained blinded to the dogs' group assignment throughout the study. A few randomization exceptions occurred for medical or welfare reasons, or following a strong request from the owner (see Section 2.4.2.1

As part of the selection criteria, dogs were examined by a licensed veterinarian to ensure that they were: 1) in acceptable general health according to gross clinical evidence, 2) intact males with no evidence of untreatable abnormalities of the reproductive organs, 3) older than 2 months of age, and 4) had no history of adverse reactions to vaccines, sedatives, anaesthetics, anti-inflammatories, antibiotics, anthelmintics, pesticides or any other products which could be used in the study.

#### 2.2. Data collection

Data collection took place during several sampling periods: 1) upon enrolment in the study between May and July 2011 (hereafter referred to as 'enrolment'), 2) between the spring/summer months of October 2011 and February 2012 (hereafter referred to as 'preintervention'), and 3) between the autumn/winter months of May and July, 2012 (hereafter referred to as 'post-intervention'). During the pre- and post-intervention data collection periods, the activities and movements of all dogs were monitored using video recordings and GPS tracking devices. Four blood samples were collected from each dog to determine their serum testosterone concentrations: 1) upon enrolment, 2) at the time of intervention (or during a concurrent 2-week period for the control group), and 3) 4 and 4) 6 months post-intervention (May and July 2012, respectively). The methodologies used to quantify serum testosterone concentrations and the

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