Pilot project: a theoretical framework for the control of fertility in a population sample of red deer from El Monte de El Pardo (Spain).

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Abstract- This study aims to promote fertility control as a non-lethal means of managing wild animal populations, specifically red deer (*Cervus elaphus*) in a region of Spain. This is the first time that a research plan on the use of immunocontraceptives in red deer in Spain has been laid. This plan is accompanied by a follow-up study to: (1) determine the effectiveness of the vaccine over 3 years in the prevention of pregnancy, (2) determine the possible contraindications, (3) identify any weaknesses and drawbacks of the treatment and (4) propose future improvements.

We expect to achieve an average inhibition of reproductive production of more than 80%, and it is hoped that in the first year the effectiveness will reach 90-100%, without notable detrimental effects on the individuals. It is expected that the treated deer will exhibit a secretion response of sufficient anti-GnRH antibodies to achieve an inhibition in their reproductive behavior and physiology and, therefore, in their fertility. Properly planned use of immunocontraceptive vaccination in red deer populations could prevent overpopulation. Significantly detrimental effects of any kind are not expected in vaccinated animals, and the use of the vaccine does not cause a severe risk to the rest of the fauna and human population. The development of this method is essential in urban and suburban areas, where lethal population control compromises animal welfare, in addition to the potential risk for human populations. We suggest that a greater effort to develop less invasive and non-lethal population control modalities is justifiable.

Index Terms- animal welfare, contraception, fertility control, immunocontraception, immunocontraceptive vaccines, red deer.

I. Introduction

Space constraints can lead to inbreeding and overpopulation for red deer (Patton et al. 2007), both having welfare implications. Overpopulation can lead to: (1) an increase in the transmission of Lyme disease (Spielman et al. 1985, Kilpatrick et al. 2014, Stein 2019), (2) a reduction in nutritional condition (Martínez and Hewitt 2001), and (3) an increase in vehicle accidents (Conover et al. 1995). Blacklegged ticks (*Ixodes scapularis*) are the main vector of Lyme disease among wild animals and human populations in eastern North America (Barbour & Fish 1993). White-tailed deer (*Odocoileus virginianus*) is a primary host for adult blacklegged ticks that feed on the blood of these animals, so the overabundance of deer is a significant determinant of Lyme disease risk (Spielman et al. 1985) and a direct relationship between deer numbers and Lyme disease cases has been shown (Kilpatrick et al. 2014). A survey revealed that Lyme disease and deer-vehicle collisions were the most prevalent concerns related to deer in suburban environments (Connelly et al. 1987, Perry et al. 2006).

The general effect of increased inbreeding in animal populations is the increase in the number of homozygous offspring, which means that these individuals can be affected by recessive or deleterious traits (Nabulsi et al. 2003). In deer, inbreeding depression is negatively correlated with lifetime and breeding success (Slate et al. 2000), therefore, it reduces the ability of populations to survive and reproduce (Jiménez et al. 1994). Inbreeding is correlated with brachygnathy (shortened lower jaw) in red deer (Zachos et al. 2007)

Recently, debates have arisen with diverse perspectives in relation to the overpopulation management of native ungulates in natural parks (Grignolio et al. 2014).

Animal populations that grow beyond certain numbers in ways that conflict with human interests (due to, for instance, disease transmissions and vehicle collisions) are sometimes regulated by humans. This is often done via control over mortality, that is, through hunting, or via control over fertility (Kirkpatrick 1999).

Some national parks have reintroduced predators such as wolves (*Canis lupus*), among other reasons to reduce the numbers of wild ungulates. However, this practice can be challenged in terms of both direct animal welfare considerations for both the translocated predators and the animals they predate (Horta 2010) and indirect ones as it can generate the opposition of farmers (Linnell et al. 2002) and hunters (Licht et al. 2010), who may react by proposing the killing of the reintroduced animals. In addition, they may be harmful for animals without being effective on high-density populations (Chair of national parks et al. 2019).

Until very recently, hunting has been the main means of reducing the numbers of animals of numerous species. However, public pressure to protect deer from hunting has increased in the last two decades as a result of growing animal ethics concerns (Turner et al. 1992; Fraser and MacRae 2011). As a result, non-lethal methods have been increasingly supported. In addition, in Monte de El Pardo, red deer populations can be considered as belonging to peri-urban or suburban areas, in which hunting, capture, or poisoning is officially prohibited for human safety reasons. The vicinity of urban and rural centers fall into the category of "security zones," in which the use of hunting weapons is prohibited or is controlled by local regulations (Mesa Gutiérrez 2017).

Recent research (e.g. Bowman, 2011; Gionfriddo et al., 2006, 2011a), has shown the need for new population management tools. An example of this is the translocation of individuals, which is still an expensive and complicated task, since it is necessary to ensure an appropriate and effective settlement of the transferred individuals. Mortality rates for relocated deer have in fact been much higher than for resident deer (O'Bryan and McCullough 1985; Craven et al. 1998).

For all these reasons, fertility control through contraception in ungulate populations has been the subject of study for almost 40 years (Kirkpatrick and Turner, 1985; Turner et al. 1992).

In early contraceptive studies in deer, steroid hormones, primarily diethylstilbestrol (DES, a synthetic estrogen) and melengestrol acetate (AMG, an active progestogen), were administered via injectable implants or orally in food(Bell 1975, Matschke 1977a, 1977b, Roughton 1979). However, implants require capture or immobilization methods that are costly, invasive, and even potentially dangerous to the animal (Turner et al. 1992).

There has recently been an increase in research on oral baits for deer in the Mediterranean bush, since factors such as season, specificity, and palatability can influence the preferences of individuals. For example, alfalfa bait can be used for oral administration of drugs or vaccines in deer(Martínez-Guijosa et al., 2017). Patton et al. (2007)reviewed the possible negative effects of steroids as hormonal contraceptives in ungulates. One of the major disadvantages of these methods is the fact that they can also act as androgens or antiandrogens, estrogens or antiestrogens, and often have glucocorticoid-like effects, exerting a wide variety of biological effects beyond their contraceptive effects (Patton et al. 2007). Critics object that oral administration of contraceptives can be unreliable, because they can be ingested by individuals who are not the subjects of the study, and they require a frequent, often daily, intake of the steroid (Turner et al. 1992). It has also been argued that it can have other effects that are not desirable because it can affect the behavior of the animals that consume it and can be passed up the food chain (Kirkpatrick and Turner, 1985; Kirkpatrick and Turner 1991).

Immunocontraception is based on the same principle used in the prevention of many diseases: a vaccine made up of proteins that cause the production of antibodies. This means that immunocontraceptive vaccines are based on the animal's ability to produce antibodies against gamete proteins, reproductive hormones and other proteins essential for reproductive activity (Kirkpatrick et al. 2011). Antibodies that the animal generates interfere with the biological activity of reproductive proteins (Talwar and Gaur 1987) and, depending on the antigen and the vaccine formulation, these can be effective from 1 to 4 years, or even longer, both from single injections and from multiple injections (Miller et al. 1999; Miller et al. 2013).

There are numerous immunocontraception procedures that interfere with the reproductive process at different levels (e.g. pituitary gland, gonads, sperm, and eggs). The main ones are classified into: PZP (Porcine Zona Pellucida) vaccines and GnRH (Gonadotropin Releasing Hormone) vaccines:

1.1. PZP vaccines (Porcine Zona Pellucida)

PZP is a combination of three zona pellucida (ZP) proteins extracted from pig ovaries. This vaccine causes the female to produce antibodies that interfere with the approach of the sperm to the egg (Florman and Wassarman 1985). After testing PZP-type vaccines in Australian opossums (*Trichosurus vulpecula*) (Duckworth et al., 1999), the Tammar wallaby (*Macropus eugenii*) (Kitchener et al., 2002), koala (*Phascolarctos cinereus*) (Kitchener et al., 2009b), and various eutherian mammals, it was concluded that the native porcine protein formed by glycosylated ZP3 is the most immunogenic of the proteins (Koyama et al., 1996; Kitchener et al., 2002). The effectiveness of the different vaccines tested is 70-80% (Moros 2015). Immunocontraception using PZP-type vaccines generates an inhibition of fertility greater than 95%, with reversible effects and without affecting females that were already pregnant, and without producing secondary effects on behavior (Kirkpatrick et al. 1990; Kirkpatrick et al. 1991) or noticeable effects on well-being (Killian et al. 2006a). It is not detected in the flesh of the animals (Miller et al. 2013). However, it has some disadvantages. First, inoculation in the female must be performed twice in 4-6 months, with administration accompanied by an adjuvant (a general immunostimulant). Second, unless the population suffers from very high mortality rated, the vaccine itself does not cause immediate or rapid population decline, although it can stabilize a deer population within 2-3 years (Kirkpatrick 1999).

1.2 GnRH vaccines

GnRH, or gonadotropin-releasing hormone, is a hormone that stimulates the release of gonadotropins from the pituitary gland, triggering the cascade of reproductive hormones (estrogen, progesterone, and testosterone) that lead to sperm production in males and ovulation in females (Miller et al. 2013).

Anti-GnRH vaccines stimulate the production of antibodies that bind to the GnRH hormone, that is, antibodies against the individual's own GnRH (United States Department of Agriculture 2007). GnRH is a neuropeptide hormone that is naturally secreted from neurons in the hypothalamus and is responsible for stimulating the synthesis and release of two fundamental hormones for proper reproductive physiology: LH (luteinizing hormone) and FHS (follicle-stimulating hormone) in the pituitary gland ("Hazum and Conn, 1988" in Baker et al., 2004). Specifically, long-term treatment with GnRH agonists prevents ovulation, by decreasing GnRH receptors on gonadotropic cells of the adenohypophysis, decreasing the sensitivity of the receptor to GnRH(Nett et al. 1975), decreasing the pituitary LH content, and suppressing the pulsatile secretion of LH and FSH into the bloodstream (Aspden et al. 1996).

To achieve this, these vaccines must be composed of numerous GnRH peptide molecules coupled to a protein (e.g. keyhole limpet hemocyanin [KLH] or blue protein [*Concholepus concholepus*]) thus forming what is known as a “conjugate”, and its application in treated animals requires an adjuvant, like PZP (Miller et al. 2013). By binding to GnRH, the antibodies reduce the ability of GnRH to stimulate the release of these sex hormones. As a result, sexual activity decreases, and animals remain in a non-reproductive state as long as there is a sufficient level of antibody activity (United States Department of Agriculture 2007).

Briefly, immunization against GnRH causes cessation of ovulation and follicular development in females (Patton et al.

2007), through the reduction of the secretion of essential reproductive hormones (Fagerstone et al., 2006; Miller et al., 2008; Miller et al., 2004). The effects on the individual are not lifelong. Following a decrease in antibody titers, natural reversion occurs in most immunized animals (Keeling and Crighton, 1984 in Patton et al., 2007), although the effects can last several years. Single-dose GnRH vaccines were effective in reducing a population of wild boars for a period of up to 36 weeks when administered to female boars during the reproductive season (Killian et al. 2003), while the response to the vaccine in males was lower (Killian et al., 2006).

This vaccine does not affect non-reproductive behavior or social organization of the species, and the protein antigens are broken down into amino acids in the gastrointestinal tract, so that the vaccine does not pass through the food chain (Miller et al. 2013). It has proven to be safe for pregnant females, without interfering with the pregnancy in deer (Miller et al., 2008), elk (*Cervus canadensis*), American bison (*Bison bison*) and wild horses (*Equus ferus caballus*) (Miller et al. 2004; Killian et al. 2008a; Powers et al. 2012).

GonaCon™ is one of the commercial brands of anti-GnRH vaccines that is most used for the control of fertility in species such as red deer. In fact, in female red deer, a single injection of *GonaCon™* has resulted in infertility for at last 1 year (Hobbs et al. 2000) and up to 2-4 years (United States Department of Agriculture 2007), with 80-100% effectiveness (Killian et al. 2008b). Additionally, *GonaCon™* significantly reduces reproductive behaviors for at least 2 years (Killian et al. 2008b), including heat cycles, which can serve as a tool for controlling the transmission of venereal diseases and diseases transmitted during childbirth (Miller et al. 2013).

In summary, the immunocontraceptive vaccination approach in wild populations is simple to administer, is effective over several years, and has little or no contraindications to treated individuals. The most recent studies show that they are safe and effective in the short term (Killian et al. 2004; Killian et al. 2006a).

For red deer, GnRH-type vaccines seem most suitable. Among all of the animal endocrine suppression modalities developed to date, GnRH immunocontraception probably poses the lowest animal welfare risks (Hampton et al. 2015). These vaccines could represent an at least partial solution to the problem of managing overpopulation in deer living near cities (Gionfriddo et al. 2006).

II. Materials and Methods

2.1 Animals

For this action plan, 200 vaccinated individuals and 50 control individuals will be captured, sedated, identified and tracked during two consecutive years. All individuals inhabit El Monte de El Pardo (Madrid, Spain, ~16,000 ha). Control animals will be administered a dose equivalent to that of the vaccine, but of a saline solution (Massei et al. 2018). The study will begin in 2021 and will have a total duration of 3 years.

As far as possible, the ages and body masses of the treated individuals will be similar, both in the treated group and in the control group (Turner et al. 1992). Female individuals of similar reproductive age will be identified following the criteria of scientific guides for hunting species (e.g. Sáenz de Buruaga et al., 2001). Furthermore, the age of each individual will be estimated by observing their dentition, specifically through tooth eruption and wear (Azorit 2011).

2.2 Vaccination

Females of reproductive age (approximately 2-10 years old) will be vaccinated (Carranza 2017). There is no disadvantage in vaccinating pregnant females, since the vaccine does not negatively impact the wellbeing of pregnant deer (Miller et al., 2008). Individuals will be vaccinated in July, as has been described in similar studies (Killian et al. 2005).

Individuals will be captured and immobilized by tranquilizer darts fired from a distance using a Pneu-Dart rifle (Model 171; Pneu-Dart, Inc., Williamsport, PA), which will contain an anesthesia mixture consisting of Telazol® (4.5 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA, USA) and xylazine hydrochloride (2.5 mg/kg; Bayer, Leverkusen, North Rhine-Westphalia, Germany) (Murray et al. 2000; Killian et al. 2005; Killian et al. 2008b; Gionfriddo et al. 2008; Gionfriddo et al. 2011b). Tranquilizer darts will be fired from authorized vehicles traveling on accessible forest roads and trails, or on foot when appropriate.

Once immobilized, individuals will be covered with a thermal blanket and undergo a veterinary examination. The evaluation of the animals will serve to detect possible symptoms or injuries compatible with diseases, wounds, and trauma. The deer will also be equipped with ear tags and radio-tracking collars (Gionfriddo et al. 2008).

The body condition of all individuals will be evaluated by palpation of the body contours because it is especially related to fat stores (e.g. Couturier et al., 2009; Finger et al., 1981; Mattiello et al., 2009; Serrano et al., 2008). Body condition will serve as a bioindicator of the health and physical condition of the treated animals.

The formula of *GonaCon™* administered will contain 1,000 µg of the GnRH peptide combined with AdjuVac™ adjuvant (Miller et al., 2008) as a 1 ml dose injection in the rump (Killian et al. 2008b; Gionfriddo et al. 2011b). *GonaCon™* must be injected intramuscularly (United States Department of Agriculture 2007) and it is expected that after a few days the vaccinated animal will begin to generate antibodies.

The inoculation with the vaccine will be administered through a single injection, since *GonaCon™* is capable of inducing contraceptive effects lasting several years with a single dose (Hobbs et al. 2000; Killian et al. 2008b; Miller et al. 2013). The immune response of individuals is variable, with effects lasting between 2 and 5 years (Madridejos 2017), or even up to 6 years (Killian et al. 2008b).

50 female deer will also be captured but not vaccinated and will serve as an untreated control group (Gionfriddo et al. 2008). Control animals will be administered a dose equivalent to that of the vaccine but of a saline solution (Massei et al. 2018).

Regular blood samples will be taken from all of the treated individuals, both control and treated groups. Blood samples will be refrigerated and taken to the regional laboratories in Madrid for analysis.

An injection of Tolazine ™ (tolazoline: 3.0 - 4.0 mg/kg; Lloyd, Inc., Shenandoah, IA) will be administered to reverse anesthetic effects and to speed recovery of individuals prior to release (Gionfriddo et al. 2008; Killian et al. 2008b; Gionfriddo et al. 2011b).

2.3 Tracing

The effectiveness of *GonaCon^{TM}* will be quantified by measuring physiological, ethological and mobility parameters, including the comparison of these data with the results of the control group in a longitudinal study.

The vaccinated individuals will be monitored by discontinuous radio-monitoring at discrete or random times during the study period. This technique is useful for determining the range of individuals and their movements (Harris et al. 1990).

Periodically every 3-4 months and until the end of the 3-year plan, vaccinated individuals and individuals from the control group will be immobilized again to collect blood samples in order to quantify progesterone and antibody levels. An evaluation of the general state of their health will be carried out, e.g. observation of possible reactions at the injection site (Massei et al. 2018), motor difficulties in the extremities, lameness, abnormal behaviors (Gionfriddo et al. 2011b) and body condition (Killian et al. 2006a). Additionally, the evaluation of females will include abdominal palpation between the months of April and May to evaluate pregnancies (Miller & Killian 2000).

Behavioral observations will be made 3 times a day at 7:30 a.m., 12:00 p.m. and 4:00 p.m. until mid-January, and then twice a day at 7:30 a.m. and 4:00 pm, at the end of February (Killian et al. 2008b). The observation periods will last 30 minutes, but will be lengthened in those cases in which reproductive activity is observed, in order to record as much information as possible for all active individuals. The criteria used to categorize reproductive behaviors will be: (1) number of sexual encounters, (2) total time of sexual activity (in days), and (3) number of heat events (Miller & Killian 2000).

2.4 Statistical analysis

Statistical analyzes will be performed comparing the data collected in the vaccinated and control group in the initial vaccination, 12 and 24 months later. Generalized Linear Mixed Model (GLMM) was used to assess the effect of GonaCon^{TM} on body condition scores and on factors that potentially affect the immune response to vaccination with GonaCon^{TM}. Treatment group, estimated age, study stage, and pregnancy status at each stage were included as fixed effects. A step-by-step backward selection based on Akaike Information Criteria (AIC) will be used to choose the optimal model (Massei et al. 2018).

III. Expected results

Using a single dose of the *GonaCon^{TM}* vaccine, we hope to find an effectiveness of in inhibiting the reproduction of females that is greater than 95% in the first year, and greater than 80% in the following two years.

Simultaneously, we expect serum progesterone levels (ng/mL) to be lower in the treated group than in the control group, since the latter will be able to go into heat and have a successful pregnancy. Additionally, the levels of anti-GnRH antibody titers are expected to be higher in the treated group than in the control group, and high enough to block reproductive physiology and consequent reproductive behaviors in vaccinated females (detectable at a dilution of 1:32,000-1: 64,000) (Quy et al. 2014). Likewise, a total or almost total absence of unwanted side effects (contraindications) derived from the injection of the vaccine in all or the vast majority of animals is expected. Aspects such as stress behaviors, variations in movement and activity, and reactions in the injection area will be evaluated during the follow-up.

IV. Discussion

Our results are expected to indicate that the vaccine is suitable for use in red deer populations in order to stabilize populations, and can be extrapolated to other species in urban, peri-urban areas and regional parks of the Iberian Peninsula, such as fallow deer and boars. Along with red deer, these populations live relatively close to human populations, and are likely to be targeted by humans.

The responsiveness of red deer to fertility control through *GonaCon^{TM}* vaccines should encourage researchers and those interested in the control of red deer or other ungulate populations to continue efforts to optimize multi-year contraceptive-inducing vaccines. Recent authors have pointed out the scarcity of scientific studies on the effects of contraceptive vaccines on the physiology and behavior of wild animals (Quy et al. 2014).

The effectiveness of the immunocontraceptive vaccine in wild red deer populations will be assessed by medium and long term follow-ups in both vaccinated individuals and those in the control group.

This population control method avoids the use of lethal force that poses a threat to humans as well the targeted animals. Likewise, controlling the number of red deer present in Monte de El Pardo will reduce the potential threats that derive directly from collisions with vehicles and transmission of diseases.

We suggest that a greater effort to develop less invasive and non-lethal population management modalities is justifiable. The current theoretical framework allows a first advance for the development of a population control that considers the welfare of individual animals.

V. Conclusions

The implementation of immunocontraception in red deer in Spain will yield results of high interest to the scientific community. In addition, it is expected that stability in the number of deer will reduce potential threats that derive directly from collisions with vehicles and transmission of diseases.

We have enough scientific evidence to believe that our results will be favorable once they are implemented in practice, which will allow us to support further projects in other territories of Spain, and probably in other countries.

Immunocontraceptive vaccine development improves over the years, and contributing to its advance represents contributing to the welfare of individual animals. For example, the latest scientific advances are moving in the direction of producing an oral species-specific GnRH vaccine bait which would replace the injections, thereby reducing the cost and the risk to the health of the animals (USDA 2007). This approach could reduce the stress of capture and the risk of anesthesia in future *GonaCon™* vaccination campaigns.

Author's contributions

Jara Gutiérrez (J.G) contributed to the study conception and design, search, compilation and revision of the literature and writing of the manuscript. Francisco Javier de Miguel (F.J. de M.) contributed to the supervision of the article and critical revision. All authors read and approved the final manuscript.

Declarations of interest

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