ACT Government

FERTILITY CONTROL OF EASTERN GREY KANGAROOS IN THE ACT

ASSESSING EFFICACY OF A DART-DELIVERED IMMUNOCONTRACEPTIVE VACCINE

MARCH 2018

Claire Wimpenny and Lyn A. Hinds

Conservation Research

Technical Report

ISBN 978-1-921117-57-2
Published by the Environment, Planning and Sustainable Development Directorate, ACT Government
Visit the EPSDD Website

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This publication should be cited as:

Wimpenny C and Hinds LA (2018). Fertility control of Eastern Grey Kangaroos in the ACT - Assessing efficacy of a dart-delivered immunocontraceptive vaccine. Environment, Planning and Sustainable Development Directorate, ACT Government, Canberra.

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Front cover: Dart vaccination of female Eastern Grey Kangaroos at Tuggeranong Homestead (L.A. Hinds).

Technical Report

Fertility control of Eastern Grey Kangaroos in the ACT - Assessing efficacy of a dart-delivered immunocontraceptive vaccine

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Conservation Research
Environment Division
Environment, Planning and Sustainable Development Directorate

March 2018

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SUMMARY

A single dose, long-lasting, remotely deliverable contraceptive would provide an additional tool for managing free-ranging Eastern Grey Kangaroo (EGK) populations. GonaCon Immunocontraceptive Vaccine has been shown to cause long-term infertility in EGKs and Tammar Wallabies following a single intramuscular injection administered by hand. If GonaCon could be administered remotely via a dart, the need to capture and anaesthetise each individual for treatment would be eliminated, making it a more efficient and practical option for use in free-ranging EGK populations.

The aims of this study are to develop and trial a dart delivery method for humanely administering GonaCon to female EGKs; to evaluate the effect of the vaccine on individuals when it is dart delivered vs injected by hand; and to evaluate if the use of GonaCon can limit the rate of increase of EGK populations.

Several dart and needle types were tested on targets and carcasses to determine the best components for administering GonaCon using a dart. Once a dart type was selected, the welfare impacts of the method were evaluated by measuring and sampling dart injection sites in EGKs 5, 30 and 120 days after treatment. The amount of bruising caused by dart vaccination was comparable to darting with anaesthetics for capture and had largely resolved by 30 days. A method of temporarily marking EGKs as they were treated was trialled, and involved one dart gun shooter firing a GonaCon dart and a second dart gun shooter simultaneously firing a marking dart filled with paint.

Female EGKs at five research sites were fitted with identification collars and treated with hand injected GonaCon (n=81), hand injected placebo (n=10) or dart delivered GonaCon (n=51). The efficacy of GonaCon was high in the year following treatment with only 13.3% of hand injected and 20.8% of dart delivered female EGKs producing a young. In the second year following treatment, none of the hand injected GonaCon treated EGKs produced a young. Data for the second year after treatment are not yet available for dart delivered GonaCon.

Annual population fecundity and annual population growth were assessed in five treated and seven untreated populations. High population fecundity rates were recorded prior to GonaCon treatment and in all untreated sites, with more than 63% of females observed with pouch young. Population level administration of GonaCon decreased the fecundity in three populations where all or most of the females had been treated with GonaCon to between 0% and 18% of females observed with pouch young in the first and second years after treatment.

The short term results obtained after only two years do not show a clear effect of GonaCon treatment on annual population growth. The annual growth rates of treated and untreated populations was variable, ranging from -35.3% to +37.1%. Two sites showed negative annual growth rates following treatment of all or most of the females with GonaCon, however, some of the untreated sites also had negative growth rates.

While the results collected so far in the study are positive, it is still not known if a single dose of dart delivered GonaCon will cause long term infertility and if this method can effectively limit the increase of free-ranging EGK populations. Ongoing monitoring of the treated individuals and populations is required to determine if an acceptable level and duration of population control can be achieved with dart delivered GonaCon.

1 INTRODUCTION

The management of wildlife populations is a complex problem faced by land managers throughout the world (Braysher 2017). Negative impacts of both native and exotic species on biodiversity, primary production, human safety and human health can trigger the need to reduce the size of problem populations (Massei and Cowan 2014). Often in these situations, lethal population control methods, such as shooting and poisoning, are utilised to reduce the damage, potential damage or likelihood of conflict caused by the species. While these methods are often an efficient and cost effective option, in some areas they are deemed unsafe or unacceptable and ongoing management is required to keep the populations at the desired level (Massei and Cowan 2014; White and Ward 2010).

Fertility control has been promoted as a potential replacement for lethal control methods, however, the circumstances where complete replacement would be possible and appropriate are limited (Bomford and O'Brien 1992; 1993; Massei and Cowan 2014). The aim of fertility control is to reduce population growth (Hone 1992; Hone 2004). It does not provide the rapid reduction in population that is often required to reduce the amount of damage or frequency of conflict. A more effective approach is to utilise the benefits of multiple techniques and to deploy fertility control into a population that has been reduced to the desired level using another method, such as shooting (Barlow et al 1997; Hone 1992; Pepin et al 2017). Used in this way, fertility control would slow the recovery of the population, thus reducing, and possibly in some cases eliminating, the need for lethal intervention in future years. A fertility control agent that is appropriate for managing wildlife species needs to be long lasting, effective when a single dose is applied, efficient to administer and have no negative side effects (Bomford 1990; Bomford and O'Brien 1992; Kirkpatrick and Turner 1991; Kirkpatrick et al 2011; Massei and Cowan 2014).

Eastern Grey Kangaroos (EGKs) are abundant across much of the Australian Capital Territory (ACT) and in some areas are present in very high densities. It is recognised that EGKs have an important role in the local environment, both through their grazing in native grassy ecosystems and by providing positive social experiences for local residents and visitors. However, in some areas of the ACT, high density populations of EGKs are causing negative environmental, economic and social impacts (ACT Government 2017a). Of particular concern is the impact of high density kangaroo populations on lowland grassy ecosystems, including the two endangered ecological communities Natural Temperate Grassland and Yellow Box-Red Gum Grassy Woodland. Several recent studies have demonstrated the detrimental effect that heavy kangaroo grazing can have on birds (Neave and Tanton 1989; Howland et al 2016a), reptiles (Dimond 2012; Howland et al 2014; Howland et al 2016b), beetles (Barton et al 2011) and ground layer plants (McIntyre et al 2010; McIntyre et al 2015). In selected ACT nature reserves, EGKs are culled annually to reduce the environmental impacts of heavy grazing. More specifically, the aim of the program is "to achieve a grazing regime that is favourable to the conservation of plants and small animals that frequent the ground layer vegetation" (ACT Government 2017a).

In ACT nature reserves, the culling technique used is shooting which is recognised as the most humane and species specific method for kangaroos (NRMMC 2008a, 2008b). Additional animal welfare conditions, such as a kangaroo shooting season and mandatory testing for all kangaroo shooters, are imposed on all kangaroo shooting activities in the ACT. High animal welfare standards are achieved in the ACT nature reserve culling program (Hampton and Forsyth 2016), however, the method does have some disadvantages. These include the need to undertake shooting on an annual

basis to maintain the population at the desired level, high costs for staff working at night, stringent safety procedures and restrictions relating to the use of high powered firearms close to urban areas, disruption to operations by protestors who oppose the killing of kangaroos, and difficult terrain in some nature reserves. The ACT Government is committed to encouraging and supporting research into alternatives to shooting. In recognition of its potential role in the management of kangaroo populations the ACT Government has supported fertility control research since 1998 (ACT Government 2017a).

Several fertility control methods have been used on EGKs. These include surgical sterilisation, hormone implants and immunocontraceptives. Surgical methods (e.g. vasectomy, castration, tubal ligation) are permanent, but are costly and invasive and require veterinary expertise. Hormonal implants are readily available and are easier to deploy but still require animals to be captured and anaesthetised to insert the implants under the skin. Two types of implants have been used in kangaroos, Levonorgestrel (synthetic steroid, progesterone) which has been shown to last for at least four years in more than 90% treated animals (Wilson and Coulson 2016), and Deslorelin (Gonadotrophin Releasing Hormone, GnRH, agonist), which lasts for 1-3 years (Herbert 2010, Wilson et al 2013, Wilson and Coulson 2016). Immunocontraceptives, or contraceptive vaccines, are administered as an intramuscular injection, again requiring capture and anaesthesia. Two types of immunocontraceptives have been trialled on EGKs, zona pellucida (ZP) (egg coat) protein vaccine which appears to require multiple doses to be effective for a length of time (Kitchener et al 2009) and GonaCon Immunocontraceptive Vaccine, a GnRH- based vaccine, which has been shown to be effective for at least eight years in more than 80% treated animals in early trials on sub-adult female EGKs (ACT Government 2017b). All currently available fertility control methods require capture and anaesthesia of each individual, a costly and resource intensive activity which limits the application of fertility control to relatively small kangaroo populations which are surrounded by barriers to kangaroo movement, such as fences, roads and urban development. For managing free-ranging kangaroo populations a more efficient delivery system, such as remote delivery using a dart, is required. Of all the fertility control agents tested in kangaroos, immunocontraceptives show the most promise for remote dart delivery because they are an injectable liquid.

For managing free-ranging kangaroo populations, GonaCon Immunocontraceptive Vaccine has shown particularly encouraging results. GonaCon has been demonstrated to provide multi-year fertility control in various eutherian species, particularly in females, including white tailed deer, bison and boar (Miller et al 2004, 2008; Fagerstone et al 2008; Massei et al 2008, 2012; Miller et al 2013), however the contraceptive effect appears to last longer in female macropodids. In the tammar wallaby, adult breeding females were infertile for at least six breeding seasons after being hand-vaccinated with a single shot of GonaCon (unpublished results, Hinds et al). Similarly a single hand-injection with GonaCon has blocked the reproductive cycle for eight years so far in a high proportion of female EGKs treated as sub-adults (ACT Government 2017b).

GonaCon is a GnRH immunocontraceptive vaccine that works by triggering an immune response which produces GnRH specific antibodies. These antibodies subsequently bind to circulating GnRH, preventing release of Follicle Stimulating Hormone and Luteinising Hormone from the pituitary gland and thereby inhibiting the normal function of the ovaries and testes. As long as sufficient antibodies are present, the treated animal will not be able to reproduce. The long lasting effect following a single dose of GonaCon is due to the formulation and preparation of the vaccine. The GnRH peptide is not naturally immunogenic, so is conjugated to a large blue mollusk protein and mixed into an

emulsion with the adjuvant, AdjuVac (Miller et al 2008). The preparation of the vaccine into an emulsion appears to be particularly important for long lasting contraception, by creating a depot effect, and producing a sterile granuloma at the injection site, the antigen is released slowly, resulting in a prolonged immune response (Miller et al 2008).

Most applications of immunocontraceptives have been as an intramuscular injection administered by hand. Dart delivery of immunocontraceptives has been trialled in white tailed deer (Turner et al 1992; Evans et al 2015) and horses (Kirkpatrick et al 1990; Naugle and Grams 2013). If GonaCon could be administered to kangaroos remotely via a dart, the efficiency of delivery would be greatly increased. Rather than having to capture, treat and monitor animals under anaesthetic, each treated individual would only need to be dart-vaccinated and followed for a short period of time to retrieve the dart after it dropped out. Once an initial level of control was established, it would be possible to manage the low level of ongoing breeding simply by adopting the practice of annually dart-vaccinating only female kangaroos with pouch young. This approach is also expected to have fewer animal welfare risks compared to capture and treatment (Hampton et al 2015).

Developing a dart delivery method for administering GonaCon to EGKs has several challenges. Firstly, the vaccine is a viscous, oily emulsion, so appropriate dart components and darting equipment that effectively expels the vaccine into the target muscle must be identified. Secondly, recognising the potential for injury caused by darting (Cattet et al 2006), the method must be humane and not cause unacceptable short or long term tissue damage. Thirdly, the method of delivery must not significantly reduce the efficacy of the vaccine. Evans et al (2015) found dart delivered GonaCon to be less effective than when hand injected, and surmised that this may be due to the vaccine and resulting sterile granulomas being more widely dispersed within the animal's tissues (subcutaneous, intermuscular as well as intramuscular) when administered via a dart. Fourth, a temporary visual marking method is required to ensure treated individuals do not receive multiple doses within a treatment session. Fifth, the method must be evaluated at a population level to determine if GonaCon can be successfully used to limit the growth rate of EGK populations.

Specifically, the aims of this project are:

> Aim 1: Develop and trial a dart delivery method for humanely administering GonaCon to female Eastern Grey Kangaroos;
>
> Aim 2: Evaluate and compare the effect of hand injected and dart delivered GonaCon on individual free-ranging female Eastern Grey Kangaroos;
>
> Aim 3: Evaluate whether use of GonaCon limits the rate of increase of free-ranging populations of Eastern Grey Kangaroos.

In 2014-15, two years of funding was granted by the ACT Government to support the research. Additional funding was secured in 2016-17 and 2017-18 to continue monitoring the treated kangaroos and sites. The project brings together researchers from within the Environment, Planning and Sustainable Development Directorate in ACT Government (Conservation Research and the Parks and Conservation Service) and CSIRO Health and Biosecurity.

This report contains results from the first three years of the project, to October 2017. Included is the development and trial of a dart delivery system for GonaCon, initial efficacy data for GonaCon injected by hand (two years of data) or administered via a dart (one year of data) to female EGKs, and initial population level results. These early results demonstrate the initial efficacy of the vaccine

and give a valuable insight into its potential as a population management tool. However, it is essential to continue monitoring the EGKs and populations to determine the long term efficacy of dart-delivered GonaCon in adult female EGKs. Without ongoing monitoring we will not be able to define how long the vaccine is effective and how this influences the growth of populations over multiple years, and it will not be possible to fully evaluate GonaCon as a population management tool for EGKs.

2 METHODS

2.1 STUDY SITES

Thirteen urban sites in the ACT with resident EGK populations were selected for the study (Figure 1, Table 1). One site (Federal Golf Course) was used for the dart delivery welfare trial in Aim 1. EGKs at five sites were treated with GonaCon to address Aims 2 and 3. To simplify the darting process and subsequent monitoring, the treatment sites were selected because they have EGK populations that are habituated to people and are easy to approach.

Kangaroos at the remaining seven sites were not treated with GonaCon and are used as a comparison for the treated populations. EGKs at three of the untreated sites have been culled for conservation reasons in the past and are likely to be culled in the future. The other four sites have never been culled.

One treatment site is completely fenced (Broadcast Australia), one partially fenced (Australian National Botanic Gardens) and the other sites are unfenced. For the evaluation of GonaCon at the population level, partially or unfenced populations were selected so that the influence of immigration and emigration could be investigated.

2.2 STUDY ANIMALS

In the ACT, EGKs are found in grassland, woodland and forest habitats, as well as modified environments such as golf courses and urban parklands. They are specialised grazers, and approximately 99% of their diet is made up of grasses and sedges (Jarman and Phillips 1989). Mating can occur at any time of year, but is most common in spring and early summer (Poole 1973, 1983; Coulson 2008). In the ACT region, the number of births peaks in summer, with a corresponding emergence of pouch young in spring (Fletcher 2007). These young at foot continue to suckle until they are approximately 18 months old. Female EGKs reach sexual maturity at approximately 18 months old and males at approximately four years old (Poole and Catling 1974). In this study, only female EGKs were treated with GonaCon because it has been demonstrated to be more effective in females than males (Snape 2012; Miller et al 2013), it is more efficient to treat only one sex, and control systems aimed at causing infertility in females are more effective than those focussing on treating males only (Bomford 1990).

Figure 1. Location of the thirteen research sites and the treatments applied at each.

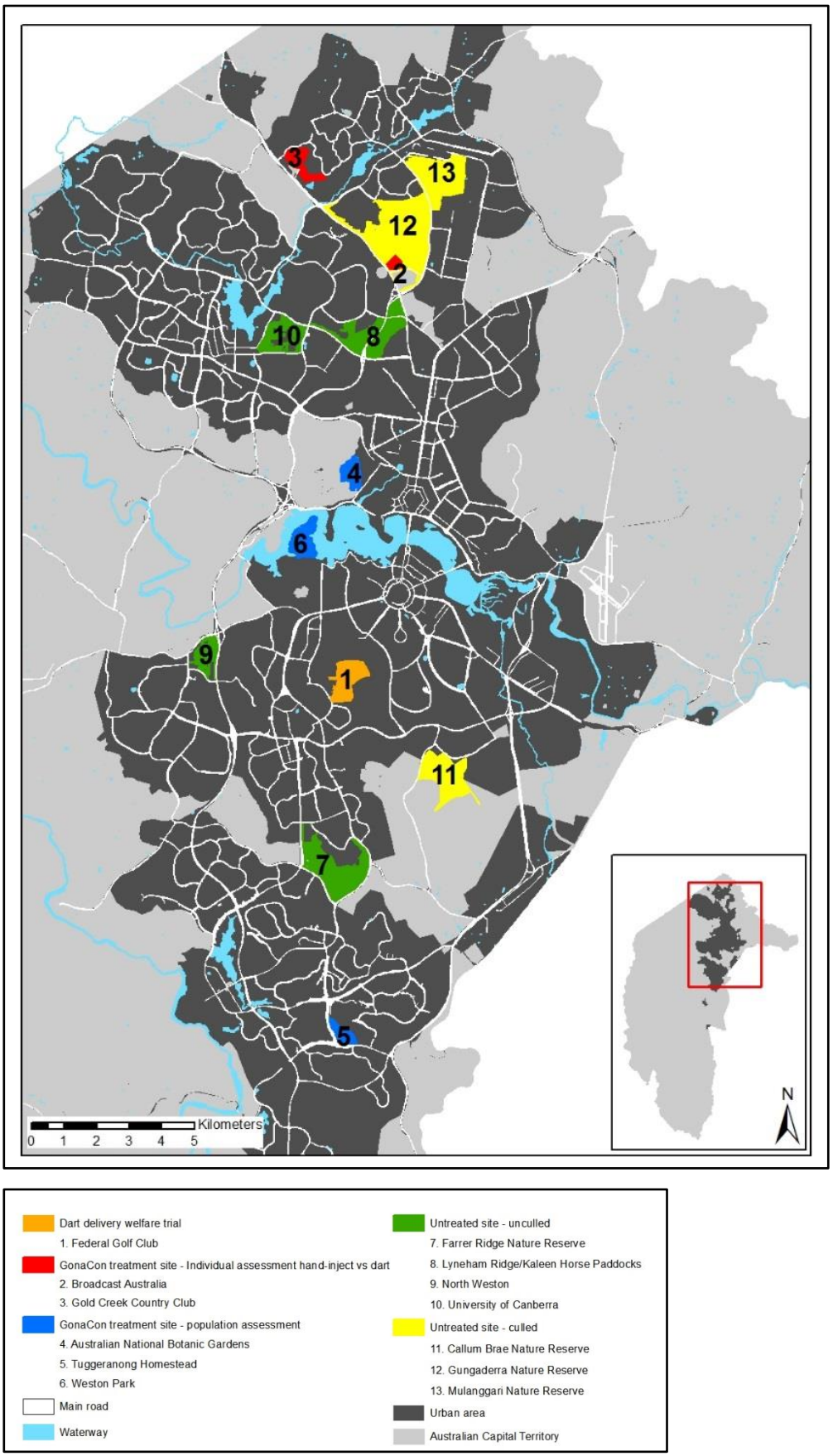


Table 1. Research site descriptions and treatments.

Site	Aim/Treatment	Description	Vegetation	Estimated EGK population (and density) at start of study
1. Federal Golf Club	Aim 1 – dart delivery welfare trial	Golf course bounded by suburb and a nature reserve, some movement of kangaroos between golf course and nature reserve, kangaroos habituated to people	Mix of irrigated grass and woodland	250 approx (2.7 EGK/ha)
2. Broadcast Australia	Aim 2 – individual assessment of hand injected vs dart delivery	Fenced site containing telecommunication towers, no public access, kangaroos not habituated to people	Native grassland with few large trees	72 (3.6 EGK/ha)
3. Gold Creek Country Club	Aim 2 – individual assessment of hand injected vs dart delivery	Golf course bounded by suburb and a nature reserve, some movement of kangaroos between golf course and nature reserve, kangaroos habituated to people	Mix of irrigated grass and woodland	108 (2.2 EGK/ha)
4. Australian National Botanic Gardens	Aim 3 – population level treatment site (individual assessment of hand-injected results used for Aim 2)	Partially fenced site, front gate open during the day to allow public access	Irrigated grass, woodland and forest gardens	23 (0.5 EGK/ha)
5. Tuggeranong Homestead	Aim 3 – population level treatment site (individual assessment of dart-delivery results used for Aim 2)	Rural block with cattle surrounded by suburbs, limited access to public	Mix of exotic pasture and woodland	35 (0.9 EGK/ha)
6. Weston Park	Aim 3 – population level treatment site (individual hand-injected results used for Aim 2)	Community park bounded by lake and suburb	Mix of irrigated grass and exotic trees/shrubs	68 (1.1 EGK/ha)
7. Farrer Ridge Nature Reserve	Aim 3 – population level untreated, unculled site	Urban nature reserve, kangaroos not culled	Native woodland	682 (3.4 EGK/ha)

8. Lyneham Ridge/Kaleen Horse Paddocks	Aim 3 – population level untreated, unculled site	Urban eucalypt plantation and horse paddocks, separated by road, kangaroos known to move between areas, kangaroos not culled	Eucalypt plantation and native/exotic grass mix	92 (0.5 EGK/ha)
9. North Weston	Aim 3 – population level untreated, unculled site	Urban open space bounded by suburbs and main road, kangaroos not culled	Mix of native and exotic grassland, woodland and exotic trees/shrubs	128 (2.2 EGK/ha)
10. University of Canberra	Aim 3 – population level untreated, unculled site	University campus with tree plantings, grassland and irrigated ovals, kangaroos not culled	Mix of irrigated and non-irrigated grass and planted native trees/shrubs	68 (0.7 EGK/ha)
11. Callum Brae Nature Reserve	Aim 3 – population level untreated, culled site	Urban nature reserve, kangaroos culled annually 2009 to 2015 and 2017, likely to be culled in future years	Native woodland	152 (1.0 EGK/ha)
12. Gungaderra Nature Reserve	Aim 3 – population level untreated, culled site	Urban nature reserve, kangaroos culled 2015 and 2016, likely to be culled in future years	Native grassland and woodland	279 (0.8 EGK/ha)
13. Mulanggari Nature Reserve	Aim 3 – population level untreated, culled site	Urban nature reserve, kangaroos culled 2013 to 2016, likely to be culled in future years	Native grassland and woodland	161 (0.9 EGK/ha)

2.3 APPROVALS AND PERMITS

All procedures in this project involving live animals were approved by the University of Canberra Animal Ethics Committee: permits CEAE 14-14 and CEAE 16-06.

The possession and use of GonaCon vaccine and a placebo/sham/control vaccine (which contained only adjuvant and buffer, no GnRH conjugate) for this project was covered by an Australian Pesticides and Veterinary Medicines Authority *'Permit to allow the possession and supply for research use of an unregistered agvet chemical product'*, permit number PER81213 issued to CSIRO Health and Biosecurity.

2.4 EXPERIMENTAL DESIGN

2.4.1 DEVELOPMENT AND TRIAL OF A GONACON DART DELIVERY METHOD (AIM 1)

Dart component selection trials

Various types of darts and needles containing an inactive GonaCon-like viscous mixture were fired at foam targets and opportunistically acquired kangaroo carcasses to determine the most appropriate components for administering GonaCon to EGKs using a dart (Table 2). Dart/needle combinations were evaluated to determine the percentage of the mixture that was expelled from the dart and the duration that the dart stayed imbedded in the target. The impact site of darts fired into carcasses was dissected to determine the depth and spread of the injected mixture.

To reduce the risk of impairing the mobility of the kangaroo during dart vaccination, the maximum desirable kinetic energy of the projectile at the point of impact was selected as approximately 12 Joules following recommendations by Friedrich (1998) for a range of ungulate species. To assess this for our darts, we fired a subset of darts through a chronograph that was set up close to the target to measure the velocity of the dart just before impact. Using the formula (Kinetic energy = 0.5 x $[\text{velocity}]^2$ x mass), we calculated the kinetic energy expended by the dart on the target. The velocity of the dart was varied by adjusting the gas pressure valve on the dart gun to establish the appropriate gas pressure required to minimise the impact force of the dart as well as maintain an acceptable level of precision for each dart type for distances from 15 to 35 metres from the target, in 5 metre increments.

To trial the visibility and longevity of potential marking media on kangaroos, various paints and dyes were painted in small patches on a piece of kangaroo fur (Table 3). After application, the approximate length of time it took for each paint or dye to dry was recorded. The paints and dyes were observed through binoculars and by the naked eye from 40 m away and were ranked from the most visible to the least visible. The fur was left outside, unprotected from rain, and observed over the following months to assess the longevity of the paints and dyes.

Table 2. Dart components tested on targets &/or kangaroo carcasses.

Brand	Injection mechanism	Dart volume	Needle width/length	Needle ports	Needle retention device
DanInject	Air pressure	1.5ml	2mm x 30mm	2 small side ports	Nil
DanInject	Air pressure	1.5ml	2mm x 30mm	2 larger side ports	Nil
Pneu-Dart, Type P	Powder charge	1ml	2.1mm x 19mm	Single port (end)	Gel collar
Pneu-Dart, Type P	Powder charge	1ml	2.1mm x 25mm	Single port (end)	Gel collar
Pneu-Dart, Type P	Powder charge	1ml	2.1mm x 19mm	Triport (1 end, 2 side)	Gel collar
Pneu-Dart, Type P	Powder charge	1ml	2.1mm x 25mm	Triport (1 end, 2 side)	Gel collar
Pneu-Dart, Injection-marker	Powder charge	1ml drug/2ml marker	2.1mm x 19mm	Single port (end)	Gel collar
Pneu-Dart, Injection-marker dart	Powder charge	1ml drug/2ml marker	2.1mm x 25mm	Single port (end)	Gel collar
Pneu-Dart, Injection-marker dart	Powder charge	1ml drug/2ml marker	2.1mm x 19mm	Triport (1 end, 2 side)	Gel collar
Pneu-Dart, Injection-marker dart	Powder charge	1ml drug/2ml marker	2.1mm x 25mm	Triport (1 end, 2 side)	Gel collar
Pneu-Dart, marker dart	Powder charge	2ml marker	Nil	Nil	Nil

Table 3. Paints and dyes tested on kangaroo fur.

Brand	Colour
Dyex Colour for Canines	Fresh Pink
Dy-Mark Spray and Mark Marking out paint	Orange
Handy Can Glo Colour water based paint	Yellow
Handycan Gloss Acrylic paint	Blue Ocean
Handycan Gloss Enamel paint	Hot pink
Heiniger Colour Flow Sheep Marker Spray	Purple
Ketchum Animal Tattoo Ink Roll-on	Green
Schwarzkopf Live Colour Ultra Brights hair dye	Green Glory
Schwarzkopf Live Colour Ultra Brights hair dye	Pillar Box Red
Steadfast Stock Mark Spray	Blue

Dart delivery welfare trial

After the completion of the dart component selection trials, a welfare trial was undertaken to assess the short term welfare impacts of dart delivery. Thirty EGKs at the Federal Golf Course were captured by firing an anaesthetic dart into the muscles of the hindquarters at a range of 15 m using a Pneu-Dart X-Caliber CO_2 powered dart rifle and Pneu-Dart 1ml Type 'P' disposable dart with 19 mm single or triport needle and dissolving gel collar. Darts contained 1ml of one of two Zoletil 100 (zolazepam hydrochloride and tiletamine hydrochloride, Virbac Australia) doses. For adult kangaroos darts contained 180 mg Zoletil, for sub-adult kangaroos darts contained 108 mg Zoletil. Each dose equates to a dose rate of approximately 6 mg/kg.

Twenty one female EGKs and nine male EGKs were used. While it is intended that GonaCon will be used only in female kangaroos, this trial was assessing the humaneness of the method, not the contraceptive effect, so the size of the animal was of interest, not the sex. Fifteen kangaroos were 'adult female size' between 30.5 kg and 41 kg and fifteen were sub-adults, between 10.5 kg and 22 kg.

Each kangaroo was assigned to one of three groups, to be assessed at 5, 30 or 120 days post darting. Within each of these groups, five (three adults and two sub-adults) were darted with single port needles and five (three adults and two sub-adults) were darted with triport needles (Table 4).

Table 4. Number of kangaroos in each assessment group of the dart delivery welfare trial.

Post darting assessment group	Needle type	Number of adult kangaroos	Number of subadult kangaroos	Total
5 days	**single port**	3	2	**5**
	triport	3	2	**5**
30 days	**single port**	3	2	**5**
	triport	3	2	**5**
120 days	**single port**	3	2	**5**
	triport	3	2	**5**
Total		**18**	**12**	**30**

Soon after the darted kangaroos were lying with their heads down, Lycra blindfolds were fitted to protect their eyes and the kangaroos were transported to a processing area where they were placed on a tarpaulin and insulating mat with a rolled hessian sack under their shoulders to promote drainage of fluid from their mouths and to maintain their airways. VHF tracking collars (Sirtrack V5C 176C VHF collar) and a coloured ear tag were fitted to each kangaroo to allow for close monitoring and efficient tracking for recapture. The location of the capture dart in the thigh was described and measured from the knee and heel so that it could be located for assessment when the kangaroo was later recaptured. Rectal temperature was monitored on all kangaroos and a blood sample (5ml) was taken from the lateral tail vein. Body measurements, weight, pouch condition and the sex and body measurements of any pouch young were recorded. Any pouch young with a head length greater than 49 mm would have been at risk of escaping and being orphaned when the female kangaroo was recaptured for reassessment so they were killed using an overdose of sodium pentabarbitone (325mg/ml, ~80mg/kg, Lethabarb, Virbac Australia). Pouch young smaller than that would still be in the pouch at the time of re-assessment and so were retained.

After processing, kangaroos were moved to a quiet recovery area and once they were able to stand they were darted from a distance of 15 m with 1 ml of GonaCon in the opposite thigh to the capture dart. The dart used was the same design as the capture dart.

After daily observation for 3-5 days following treatment, the kangaroos were monitored weekly by checking the VHF collar frequencies for any mortality signals and observed visually fortnightly.

At three intervals after darting – 5, 30 and 120 days – 10 kangaroos were located using their VHF collars and capture-darted. Capture darts targeted the thigh on the same side as the original capture dart so as not to interfere with the GonaCon dart site. The kangaroos were transported to a processing area and following the collection of blood (5ml) from the lateral tail vein or jugular, they were killed using an overdose of sodium pentabarbitone (325mg/ml, ~80 mg/kg, Lethabarb, Virbac Australia) injected intracardially or intraveneously. Adult weight and body parameters were again measured. Any pouch young were measured, sexed and killed using an overdose of sodium pentabarbitone (325mg/ml, ~80 mg/kg, Lethabarb, Virbac Australia) injected intracardially.

The GonaCon dart site and original capture dart site were located and dissected. Photographs and measurements were taken of the external skin surface, subcutaneous area, and muscles. Measurements of the extent of bruising and other damage to tissues was undertaken and

representative samples were fixed in 10% phosphate buffered formalin. For the GonaCon dart sites, the location, spread and depth of the vaccine was measured and described.

Subsequently, samples were blinded and a representative subsample were submitted to a pathologist for histopathological assessment.

2.4.2 EFFECT OF GONACON ON INDIVIDUALS AND POPULATIONS (AIMS 2 AND 3)

Aim 2 – Evaluate and compare the effect of hand injected and dart delivered GonaCon on individual free-ranging female Eastern Grey Kangaroos.

At two sites (Broadcast Australia and Gold Creek Country Club) (Table 1), wild female EGKs were collared and treated with GonaCon injected by hand, placebo injected by hand, or GonaCon administered remotely via a dart as described below in *Kangaroo capture, processing and treatment*.

Subsequently the breeding status of each individual was monitored to assess contraceptive effectiveness and compare the efficacy of the two delivery methods.

Aim 3 – Evaluate attempts to limit the rate of increase of free-ranging populations of Eastern Grey Kangaroos using GonaCon.

At three sites (Australian National Botanic Gardens, Tuggeranong Homestead and Weston Park) (Table 1), as many female EGKs as possible were treated with GonaCon to assess the effectiveness of the vaccine in suppressing population growth at the population level. In the first year at each site, female EGKs were captured, collared and treated with GonaCon via hand injection or dart delivery as described below in *Kangaroo capture, processing and treatment*. EGKs were fitted with identification collars so that they could be included in the individual efficacy assessment in Aim 2. In subsequent years, any new breeding female kangaroos (which included young kangaroos that had commenced breeding in the previous 12 months, new kangaroos to the site, or adult kangaroos not treated in the previous year) in each population were treated with dart delivered GonaCon. Kangaroos dart-vaccinated in subsequent years were not fitted with identification collars and will not be individually identifiable in future years. Annual population estimates and fecundity assessments (details below) were conducted in the treated populations and untreated populations for comparison.

Kangaroo capture, processing and treatment

EGKs were captured by firing an anaesthetic dart into the muscles of the hindquarters at a range of 15-40m (Figure 2a) using a CO_2 powered dart rifle (Pneu-Dart X-Caliber or DanInject JM.SP.25) and powder charge (Pneu-Dart 1ml Type 'P' disposable dart with 2.1mm x 19mm single or triport needle and dissolving gel collar) or air-pressurised darts (DanInject 1.5ml reusable dart, 1.5mm x 25mm dual port needle with metal collar). Darts contained 1 ml of one of four drug mixes (Table 5). There is currently no standard protocol for anaesthesia of EGKs. Individual veterinarians use their discretion in the choice of anaesthetics and dose rates. The number of kangaroos captured in this project provided an opportunity to trial different drug mixes to evaluate the time to and quality of induction, the depth of anaesthesia, and length and quality of recovery. Results from this comparison have not yet been analysed and will be reported separately.

Table 5. Drug mixes and dose rates contained in anaesthetic darts.

	In dart		Injected by hand once recumbent	Injected by hand approx 1hr after dart (reversal for Medetomidine)
Drug Mix	**Zoletil**	**Medetomidine**	**Azaparone**	**Atipamezole**
Zol	5-7mg/kg			
HiZLoM	4-5mg/kg	18-20ug/kg		75ug/kg
LoZHiM	1.5-2mg/kg	0.07mg/kg		0.35mg/kg
Zol + Azap	5-7mg/kg		1.5mg/kg	

Zoletil = tiletamine hydrochloride and zolazepam hydrochloride, Zoletil 100, Virbac Australia
Medetomidine = medetomidine hydrochloride, Ilium Medetomidine, Troy Laboratories
Azaparone = Stresnil, Elanco Animal Health
Atipamezole = atipamezole hydrochloride, Ilium Atipamezole, Troy Laboratories

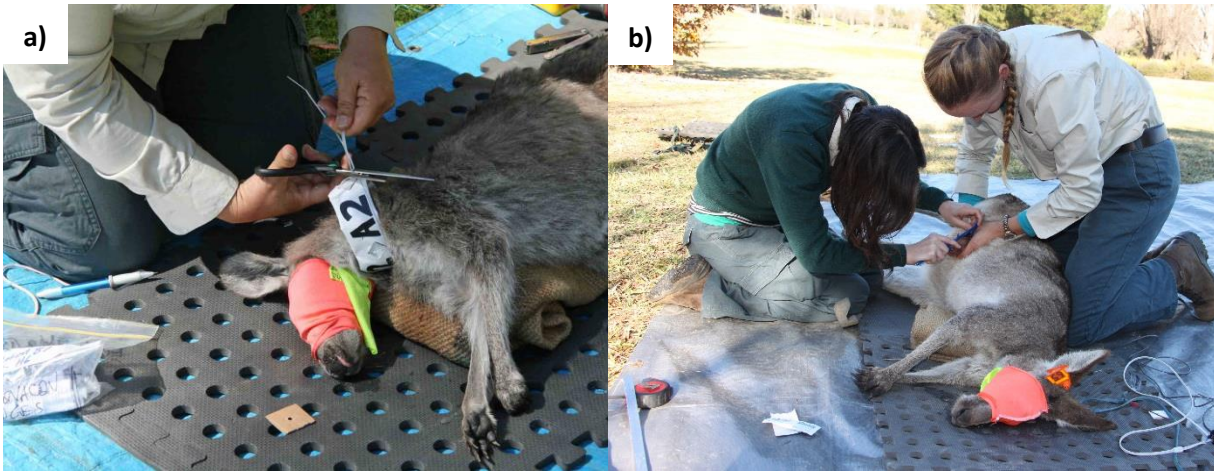
Soon after the darted kangaroos were lying with their heads down, Lycra blindfolds were fitted to protect their eyes (Figure 2b). The kangaroos were transported to a processing area where they were placed on a tarpaulin and insulating mat with a rolled hessian sack under their shoulders to promote drainage of fluid from their mouths and to maintain their airways (Figure 3a). Rectal temperature was monitored on all kangaroos and pulse, respiration and blood oxygen level were measured on a subsample of kangaroos using a pulse oximeter (Darvall H100).

Figure 2. Kangaroo capture. Kangaroos were captured with darts containing anaesthetic drugs (a), and once they became unresponsive were fitted with Lycra blindfolds to protect their eyes. Blindfolds remained in place until the animal was sufficiently conscious to remove it (b).

Whilst anaesthetised, kangaroos were fitted with individually labelled vinyl identification collars and a coloured ear tag (Figure 3a and b). Body measurements, weight, pouch condition and the sex and body measurements of any pouch young were recorded. Animals allocated to the hand-injected treatment were injected with 1ml GonaCon (1000µg) or placebo dose (1ml) into the rump muscle on the opposite side to the capture dart site. Animals allocated to the dart delivery treatment group were left untreated at this time.

Following processing and treatment, the kangaroos were moved to a quiet shady recovery area where they were monitored until they regained consciousness and moved away.

Figure 3. Kangaroo processing. The anaesthetised kangaroos were placed on a tarpaulin and foam mat with a rolled hessian sack under their shoulders to maintain drainage from the muzzle. Kangaroos were each fitted with a vinyl identification collar (a) and coloured ear tag (b).



Two to 12 months after initial capture and collaring, 1ml of GonaCon (1000 µg) was administered remotely to the dart delivery treatment group using a CO_2 powered dart rifle (Pneu-Dart X-Caliber) and powder charge dart (Pneu-Dart 1ml Type 'P' disposable dart with 19mm single or triport needle and dissolving gel collar). A second dart gun shooter simultaneously fired a marker dart (Pneu-Dart 2ml marker dart) containing 1ml of paint at a subset of these kangaroos to trial a temporary marking system. The kangaroos were approached in a vehicle or on foot and darted from a distance of 15-35m. Once darted with GonaCon, the kangaroos were observed until the dart dropped out so that it could be recovered and to confirm that all vaccine had been expelled. Dart vaccinated kangaroos were observed several times over the following six weeks to check for any effects of the dart delivery on mobility and overall condition and to record the duration that marking paint was still visible on the fur.

Breeding and welfare checks

Research sites were visited every 6-8 weeks to observe each individual and to check visually for signs of young in the pouch. Observations were made from a vehicle or on foot using binoculars and spotting scopes. Where it was possible to confirm associations between research kangaroos and a young at foot, these were also recorded.

Kangaroo population estimates

Population estimates were undertaken annually in winter 2015 to 2017 at each of the research sites using one of three methods (Table 6). While Broadcast Australia and Gold Creek Country Club are being used to assess the effect of GonaCon on individuals (Aim 2) and there are many untreated females in these populations, annual population estimates and fecundity assessments were still undertaken at these sites as this is likely to provide some preliminary information about the level of population control that is achieved by treating a lower proportion of the females in the population.

Direct counts were used in small, open sites. This involves one to three people on foot or in vehicles coordinating their movements via radio in order to conduct a 'head count' of all of the kangaroos at the site.

Sweep counts involve a coordinated group of people walking through a site and counting the kangaroos as they move through the line of walkers (Coulson & Raines 1985). The execution of this method is site dependent and may involve techniques to discourage kangaroos to move away from surrounding roads, such as having two lines of people moving from opposite ends of the site to meet in the centre.

Direct and sweep counts were repeated at least once at each site and the results were deemed acceptable if the number of kangaroos recorded in each count varied by less than 10%.

Walked Line Transect counts were undertaken in larger, more heavily wooded areas where direct or sweep counts were not practical or feasible. Walked Line Transect counts are one of the Distance Sampling methods (Buckland et al 2007) and involve one person using a laser rangefinder and voice recorder to record the distance, bearing and number of individuals in each group of kangaroos observed while walking along predetermined transects. The surveys were undertaken within the first three hours after sunrise on multiple days and approximately 40km of transects were walked in each site in total. Results were analysed using Distance 6.2 to estimate the kangaroo population at the site.

Table 6. Method used for estimating the kangaroo population at each research site

Site	Count method
Broadcast Australia	Direct Count
Gold Creek Country Club	Direct Count
Tuggeranong Homestead	Direct Count
Weston Park	Direct Count
University of Canberra	Direct Count
Mulanggari Nature Reserve	Direct Count
Australian National Botanic Gardens	Sweep Count/Direct Count
North Weston	Sweep Count
Lyneham Ridge/Kaleen Horse Paddocks	Sweep Count
Gungaderra Nature Reserve	Sweep Count
Farrer Ridge Nature Reserve	Walked Line Transect Count
Callum Brae Nature Reserve	Walked Line Transect Count

Population level fecundity assessments

Population level fecundity was assessed at the five treatment sites and four untreated sites (Farrer Ridge, Lyneham Ridge/Kaleen Horse Paddocks, North Weston and University of Canberra) (Table 1) in late September/early October 2015 to 2017 by recording the proportion of adult females with pouch young. Assessments were made at three additional untreated sites (Callum Brae, Gungaderra and Mulanggari) in 2016 and 2017 (Table 1). Due to the seasonal breeding of kangaroos in this region, at this time of year most females have a large pouch young that is easily observed from a distance (Fletcher 2007, Nave et al 2002). Groups of kangaroos were observed through binoculars and each individual was classified as adult male, adult female with pouch young, adult female without pouch young or sub-adult.

Opportunistic collections of tissues from killed kangaroos

Since the start of the experiments (Aims 2 and 3), a number of collared kangaroos have been killed through misadventure (such as vehicle collision near or on treatment sites). Where possible, information was gathered on the general condition of the animal, pouch condition and presence of young, the pathology of the GonaCon injection site, and the status of the reproductive tract (active or inactive based on gross morphology).

3 RESULTS

3.1 Development and trial of a GonaCon dart delivery method

3.1.1 DART COMPONENT SELECTION TRIALS

The Pneu-Dart Type P 1ml dart with 2.1mm x 19mm needle fitted with a dissolving gel collar was identified as the dart/needle combination that performed the strongest in the dart selection trials and was chosen to be trialled on live kangaroos (Table 7). Both the 19 mm and the 25 mm needles injected the GonaCon-like mixture into the muscle but also deposited some subcutaneously. The shorter needles were chosen because they reduce the risk of injury to bones or organs if the dart misses the target muscle. No difference in performance was apparent in these trials between single and triport needles. However, accurate measurements of the depth and spread of the GonaCon-like mixture was difficult because the deposit was often displaced when the carcass muscle was dissected. Both single and triport needles were used in subsequent trials on live animals in case a difference in granuloma formation or vaccine efficacy did exist.

At distances up to 25 m, the average impact force measured for the Pneu-Dart Type P 1ml darts loaded with the GonaCon-like mixture was less than 12 Joules (range 9.5 – 10.9). However, at greater distances, from 30 to 35m, the average impact forces required to maintain appropriate levels of precision of the darts were 15.9 (range 13.8 – 18.5) and 17.8 (range 16.5 – 20.3) Joules respectively.

Pneu-Dart injection–marker darts were not selected for use in trials on live kangaroos in this study because sufficient precision could not be achieved at any distance without darts striking the target at greater than 12 Joules, even when filled with 1ml of GonaCon-like mixture and only 1ml of marking paint (rather than 2ml of marking paint). The Pneu-Dart 2ml mark-only dart containing 1ml of marking paint was selected for subsequent marking trials. This dart would be simultaneously fired at the target kangaroo by a second dart gun shooter.

Previous experience indicated that plain needles (without any retention device) often bounce out of the animal on impact or shortly after and can result in incomplete drug injection. Whilst Dan-Inject darts and needles successfully injected the GonaCon-like mixture on a majority of occasions with impact force of 12 Joules or less, the needles are only available as plain or with metal retention devices (collars or barbs) that require manual removal from the animal (and hence are not suitable for dart delivery of GonaCon). The Pneu-Dart needles with dissolving gel collars were thus chosen over the Dan-Inject equipment.

Table 7. Assessment of dart/needle types tested on targets and kangaroo carcasses

Dart description	Dart remained embedded in target?	Vaccine injected into muscle?	Vaccine 100% expelled?	Measured impact force ≤12 Joules for distances up to 25m
Pneu-Dart, Type P 1ml dart Needle: 2.1mm x 19mm; 2.1mm x 25mm Ports: triport; single port Needle retention device: dissolving gel collar	YES	YES – shorter 19mm needles less likely to injure bone or organs if target muscle is missed	YES	YES
Pneu-Dart, 2ml marker dart No needle Filled with 1ml of marking paint	N/A	N/A	N/A	YES
Pneu-Dart, 1ml Injection- 2ml Marker dart Needle: 2.1mm x 19mm; 2.1mm x 25mm Ports: triport; single port Needle retention device: dissolving gel collar	YES	YES	YES	NO
Dan-Inject, 1.5ml dart Needle: 2mm x 30mm Ports: dual port standard; dual port larger Needle retention device: none	YES – but lack of needle retention device expected to cause darts to bounce out when used on live animals	YES	YES – larger ports more reliable than smaller	YES

3.1.2 TEMPORARY MARKER

Of all the dyes and paints tested on kangaroo fur (Table 4), the Yellow Handy Can Glo Colour water based paint, Ocean Blue Handycan Gloss Acrylic paint and Orange Dy-Mark Spray and Mark Marking out paint were most visible from 40 m with and without binoculars seven days after application and after being exposed to rain (Figure 4). The orange spray paint was more difficult to see on wet fur and rubbed off more when touched, so the yellow and blue paints were chosen to trial on live kangaroos.

As part of the dart vaccination process in July 2016, attempts were made to fire a marker dart at 40 kangaroos which were simultaneously being darted with GonaCon. The marker dart hit the kangaroo on 23 (58%) occasions and left a visible mark on the leg, back or tail. On one occasion when the marker dart hit the kangaroo but the GonaCon dart did not, the individual was followed and subsequently darted with GonaCon. The mark left by the paint was small and often hard to see with binoculars. Yellow paint was more visible than blue. Paint was visible on 55% of the collared marked animals 7-16 days after darting and only 20% had visible paint marks after 35-38 days. Inspection of a kangaroo that was killed by a motor vehicle two weeks after darting revealed a paint mark (Figure 5), despite this not being observed using binoculars a week previously. Paint marks were also observed on some individuals in the later checks that were not observed in earlier checks. This indicates that the longevity of the paint may be greater than observed, but the size of the paint mark was often too small to see from a distance.

Figure 4. Some paints and dyes tested on kangaroo fur. The fluoro yellow and blue were most visible from 40 m with and without binoculars on wet and dry fur.

Figure 5. Paint marker on roadkill kangaroo two weeks after darting.

In August 2017, seven uncollared female kangaroos with pouch young were dart vaccinated at Weston Park (n=5) and Tuggeranong Homestead (n=2). Again, attempts were made to simultaneously mark these individuals; 71% (5/7) of the kangaroos were successfully marked with yellow paint (Figure 6). However, the marker dart initially missed on two of these occasions so these individuals were followed and subsequently marked.

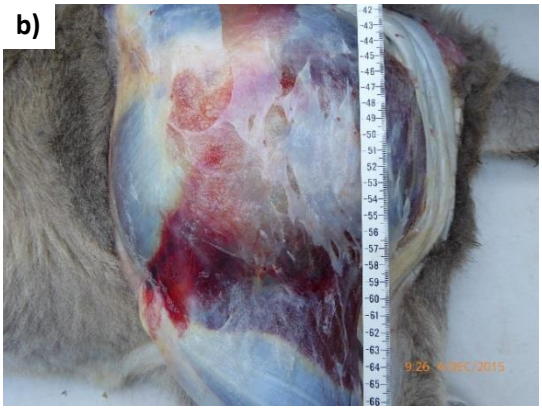
Figure 6. A female kangaroo at Weston Park after simultaneous dart vaccination and marking by two dart gun shooters in 2017. Yellow marker paint is visible below the pink dart. Once the gel collar on the needle melts, the dart drops from the animal and can be collected for disposal.

3.1.3 DART DELIVERY WELFARE TRIAL

Visual inspection and measurements of the darting locations at necropsy confirmed that GonaCon vaccine was successfully delivered via the dart. However, unlike hand-injected vaccine, the dart-injected GonaCon was more dispersed being variously deposited subcutaneously, under the fascia, in the muscle and in between the muscles of the hindquarters. At 5 days post darting, both the capture dart site and the GonaCon dart site showed areas of mainly superficial bruising (Figure 7a and b). At 30 days post darting the bruising had resolved in a majority of the GonaCon dart and capture dart locations (Figure 7c and d). The GonaCon sites were characterised by fibrotic tissue containing many granulomas (characteristic of vaccine deposits). Fibrotic tissue (not containing granulomas) was also present at the capture dart sites. At 120 days, the fibrotic tissue associated with the GonaCon dart site was softer, had reduced in area and was more vascular (Figure 7e) and granules of vaccine could be identified throughout the darted site. In contrast, the capture dart site was hard to locate in some animals by 120 days (Figure 7f). No difference in the amount of bruising or size and depth of the vaccine deposit was found between the single port or triport needles.

Histopathological assessment of a subsample of the tissues collected found no infection in any of the samples. Detailed analysis of the tissue samples and analysis of blood samples has not yet been conducted, and will be reported separately.

Figure 7. Subcutaneous view of GonaCon and capture dart sites from the dart delivery welfare trial: a) and b) 5 days post darting; c) and d) 30 days post darting; e) and f) 120 days post darting.

	GonaCon Dart	Capture Dart
5 days	a) 	b) 
30 days	c) 	d) 
120 days	e) 	f) 

3.2 EFFECT OF GONACON ON INDIVIDUALS AND POPULATIONS

3.2.1 NUMBER OF ANIMALS TREATED

Between June 2015 and May 2016, 148 female EGKs across five sites were captured, measured, fitted with identification collars and ear tags and treated with hand injected GonaCon, hand injected placebo or released untreated for later dart delivery (Table 8).

Table 8. Number of female kangaroos fitted with identification collars and treated with GonaCon or a placebo at each research site (June 2015 – July 2016)

Site	Hand Injected GonaCon	Hand injected Placebo	Dart Delivered GonaCon	Collared for Dart Delivery but died prior to treatment	Died during Dart Delivery	Collared for Dart Delivery but not treated	Total
ANBG	10						10
Broadcast Australia	10	5	13	2		1	31
Gold Creek Country Club	26	5	21				52
Tuggeranong Homestead			17	2	1		20
Weston Park	35						35
Total	**81**	**10**	**51**	**4**	**1**	**1**	**148**

At Gold Creek Country Club, the hand injected treatments were administered in two groups. In September 2015, ten adult females were treated with hand injected GonaCon and five adult females were treated with a placebo injection. In April 2016, 16 sub-adult females were treated with hand injected GonaCon. The efficacy results for these two age groups are presented separately.

In July 2016, 51 collared and previously untreated EGKs across three sites (Broadcast Australia, Gold Creek Country Club and Tuggeranong Homestead) were administered with GonaCon via a dart (Table 8, Figure 8). Three uncollared kangaroos with pouch young at Weston Park were also administered dart delivered GonaCon as part of the population level control at that site.

In August 2017, a further five uncollared kangaroos with pouch young at Weston Park and two uncollared kangaroos with pouch young at Tuggeranong Homestead were administered dart delivered GonaCon.

Figure 8. Dart vaccination of female kangaroos fitted with identification collars and ear tags. The centre kangaroo has just been darted with GonaCon. Once the vaccine is expelled, the pink dart drops from the animal and is collected for disposal.

3.2.2 FATE OF KANGAROOS

As of October 2017, of the 148 kangaroos fitted with identification collars and ear tags (Table 8), 93 kangaroos remain alive at the research sites, 23 have been missing from their site since at least June 2017 and 32 are confirmed to have died (Table 9). Of the 16 missing EGKs from Gold Creek Country Club, 12 were last seen in May/June 2017 and appear to have dispersed from the site at a similar time.

Table 9. Status of collared research kangaroos October 2017.

Site	Alive	Confirmed dead	Missing	Total
Australian National Botanic Gardens	4	4	2	10
Broadcast Australia	25	5	1	31
Gold Creek Country Club	27	9	16	52
Tuggeranong Homestead	17	3	0	20
Weston Park	20	11	4	35
Total	**93**	**32**	**23**	**148**

When kangaroos were being selected for collaring and treatment, one animal was euthanased due to a capture injury. This individual has not been included as a research animal in Table 8 or 9.

Table 10. Cause of death / reason for euthanasia for kangaroos that died unexpectedly. Note: this table includes the additional animal that was euthanased due to capture injury not included in Tables 8 or 9.

Cause of death/reason for euthanasia	Number
Motor vehicle collision	19
Unknown - necropsy not possible	6
Poisoned by plant	3
Injured during darting	2
Died during anaesthesia recovery	1
Found in lake and euthanased	1
Illness/other injury	1
Total	**33**

Of the 33 animals that have died, 19 were killed in motor vehicle collisions. Most of these were at or near to their site, however, six kangaroos were killed on roads between 2 and 6 km away from their treatment site. One kangaroo from Weston Park required euthanasia following being pulled from Lake Burley Griffin. One kangaroo was euthanased after being found in poor condition and unable to stand. Necropsy found fluid in the lungs, an enlarged heart and fluid in the swollen area of the right foot, but no active infection or injury. This animal had been hand vaccinated with GonaCon and examination of the site of injection revealed a typical contained area of vaccine within the muscle block of the left hind leg. There was no infection in this area. Six kangaroos died of unknown causes. The carcasses of these individuals were too decomposed or scavenged to conduct meaningful necropsies.

During a population count at ANBG in August 2016, three research kangaroos were found dead in close proximity to each other. Samples of their stomach contents were collected to test for suspected poisoning. All three samples were positive for monofluroacetate, the active ingredient in 1080 poison and found to occur naturally in *Gastrolobium* species from Western Australia. ANBG has over 30 species of *Gastrolobium*, some that were flowering or seeding at the time the kangaroos were found dead. Commercial 1080 poison baits were not in use at the time at the site.

Three kangaroos died as a result of darting injuries (n=2) or overheating during recovery from anaesthesia (n=1). Protocols are in place to reduce the chance of injury and hyper- or hypothermia during darting and recovery from anaesthesia. These include appropriate training and licencing of staff, using the smallest, lightest darts possible and short needles, limiting the impact force of the darts, not darting on hot days, monitoring body temperature and cooling animals with water or warming with hot water bottles if required, and placing them in shady areas to recover. However, accidents do occur and, as with all anaesthesia, there is a chance an individual will suffer an adverse reaction. The number of deaths caused by darting or anaesthesia is low compared to the expected mortality rate of 5-10% suggested for kangaroos by Tribe et al. (2014) – with 3 deaths from 216 darting events (1.4%).

3.2.3 DISSECTION OF GONACON INJECTION SITES AND REPRODUCTIVE TRACTS

Opportunistic injection site dissections were possible for 21 kangaroos which died through misadventure during this study (see Section 3.2). Of these kangaroos, 17 had been hand injected with GonaCon. The injection sites typically contained a single deposit of vaccine that had formed multiple sterile granulomas (Figure 9). No infection was observed at any of the vaccine sites. In 70.6% (12/17) of cases the area of vaccine was completely contained within the muscle block of the hind leg. In 23.5% (4/17) cases, the majority of the vaccine was contained within the muscle block, but a small amount (10% or less) was present subcutaneously or below the fascia layer above the muscle. In 5.9% (1/17) cases half of the vaccine was within the muscle and half was found between muscle blocks.

Four of the kangaroos that died had been treated with GonaCon administered by a dart. The vaccine site was located in three of these individuals. Compared to hand injection, the vaccine deposit was more dispersed and more was present in the subcutaneous and fascia layers, rather than being predominantly contained within the muscle. In one case, all of the vaccine was present subcutaneously and below the fascia layer above the muscle. In the other two cases, 90% and 40% of the vaccine was present within and between the muscles, with the remainder deposited subcutaneously and in the fascia layers. No infection was found at any of the dart delivered vaccine sites. Despite thorough dissection, the vaccine site could not be located in the fourth dart delivered kangaroo. At the time of treatment, the dart was seen to hit this individual but fell out immediately. The vaccine fully expelled from the dart and there was fur on the needle, so the treatment appeared to have been successful, however, based on the dissection, it appears that the vaccine may not have injected into the kangaroo. This conclusion is supported because this individual had successfully bred after dart vaccination, and at the time of death a corpus luteum was present on one ovary, indicating she had ovulated subsequently.

In all other cases where the reproductive tract was examined (n=13), the tracts and ovaries were small and appeared inactive based on gross morphology, including those of two hand injected individuals which had successfully bred once after treatment.

Figure 9. Contained, sterile, injection site reaction with granulomas typical of GonaCon hand injection.

3.2.4 EFFICACY OF HAND INJECTED AND DART DELIVERED GONACON

A summary of the percentage of female EGKs with young in each treatment group prior to treatment (for dart delivery animals), at the time of treatment and following treatment is presented in Table 11.

Table 11. Percentage of female EGKs with young in each treatment group per year. **([1] Year prior to treatment).**

Treatment group	Year of treatment	Collaring[1]	Year 0 (Treatment)	Year 1	Year 2
Hand injected GonaCon	2015		92.3% *(n=65)*	13.3% *(n=60)*	0% *(n=47)*
Hand injected Placebo	2015		100% *(n=10)*	90% *(n=10)*	88.9% *(n=9)*
Dart delivered GonaCon	2016	92.2% *(n=51)*	96.1% *(n=51)*	20.8% *(n=48)*	
Hand injected GonaCon (sub-adults)	2016		0% *(n=16)*	0% *(n=10)*	

At the time of treatment in 2015, 92.3% (60/65) of the EGKs hand injected with GonaCon and 100% (10/10) of the EGKs hand injected with the placebo had a pouch young or young at foot. In the year following treatment, only 13.3% (8/60) of the kangaroos treated with hand injected GonaCon produced a young. This low reproductive rate was consistent across sites, ranging from 0% (0/9) at ANBG to 30% (3/10) at Gold Creek Country Club. In the second year following treatment, none (n=47) of the hand injected GonaCon treated kangaroos produced a young (Figure 10). By comparison, 90% (9/10) of those treated with the hand injected placebo successfully bred in the year following treatment and 88.9% (8/9) successfully bred in the second year (Figure 11).

None of the 16 sub-adult female EGKs treated with hand injected GonaCon at Gold Creek Country Club in 2016 produced a young in the year following treatment.

When initially captured and fitted with identification collars (the year prior to treatment), 92.2% (47/51) of the dart delivery treatment group had a pouch young or young at foot. At the time of GonaCon dart delivery, 96.1% (49/51) of the EGKs had a pouch young. In the year following treatment, only 20.8% (10/48) of the dart delivery EGKs produced a young (Figure 12). The number of young produced by animals treated with dart delivered GonaCon was low on all sites, ranging from 16.7% (2/12) at Broadcast Australia to 23.5% (4/17) at Tuggeranong Homestead.

Figure 10. Percentage of female EGKs with young at the time of treatment and following treatment with GonaCon administered via hand injection. The total number of EGKs assessed each year is presented above bars. **(ANBG = Australian National Botanic Gardens, BA = Broadcast Australia, GCCC = Gold Creek Country Club, WP = Weston Park, Total = all sites combined).**

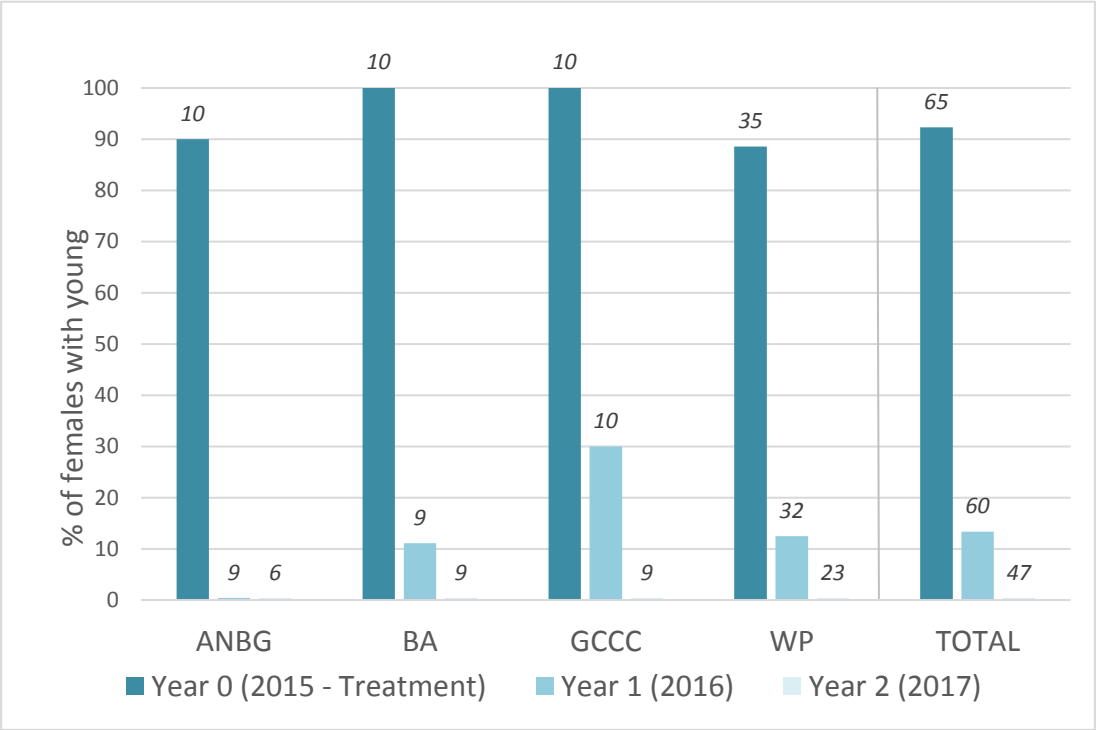


Figure 11. Percentage of female EGKs with young at the time of treatment and following placebo treatment administered via hand injection. The total number of EGKs assessed each year is presented above bars. **(BA = Broadcast Australia, GCCC = Gold Creek Country Club, Total = both sites combined).**

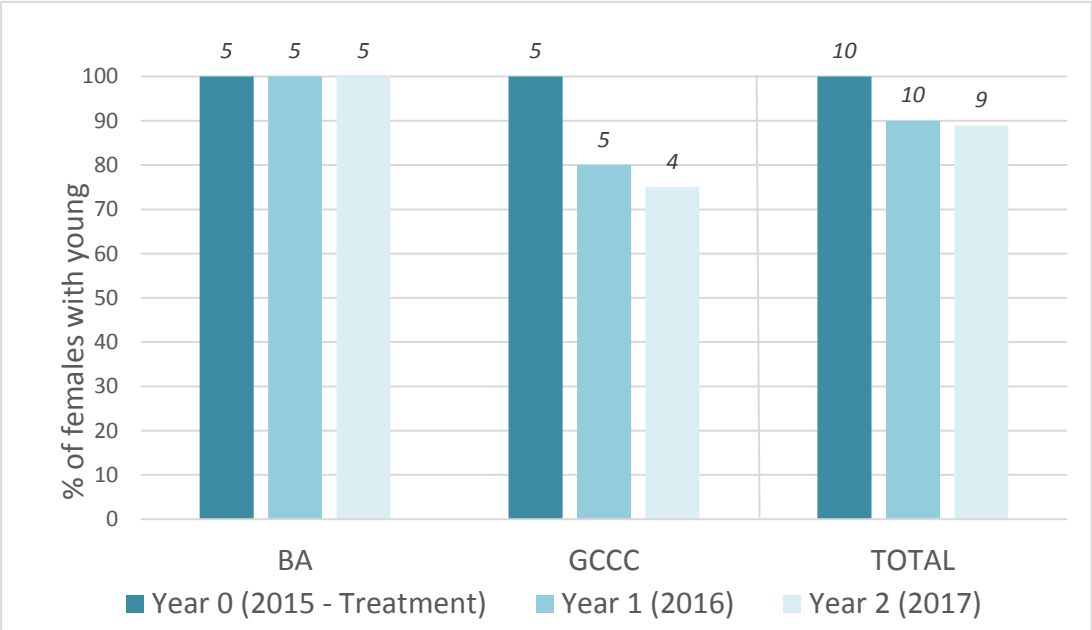
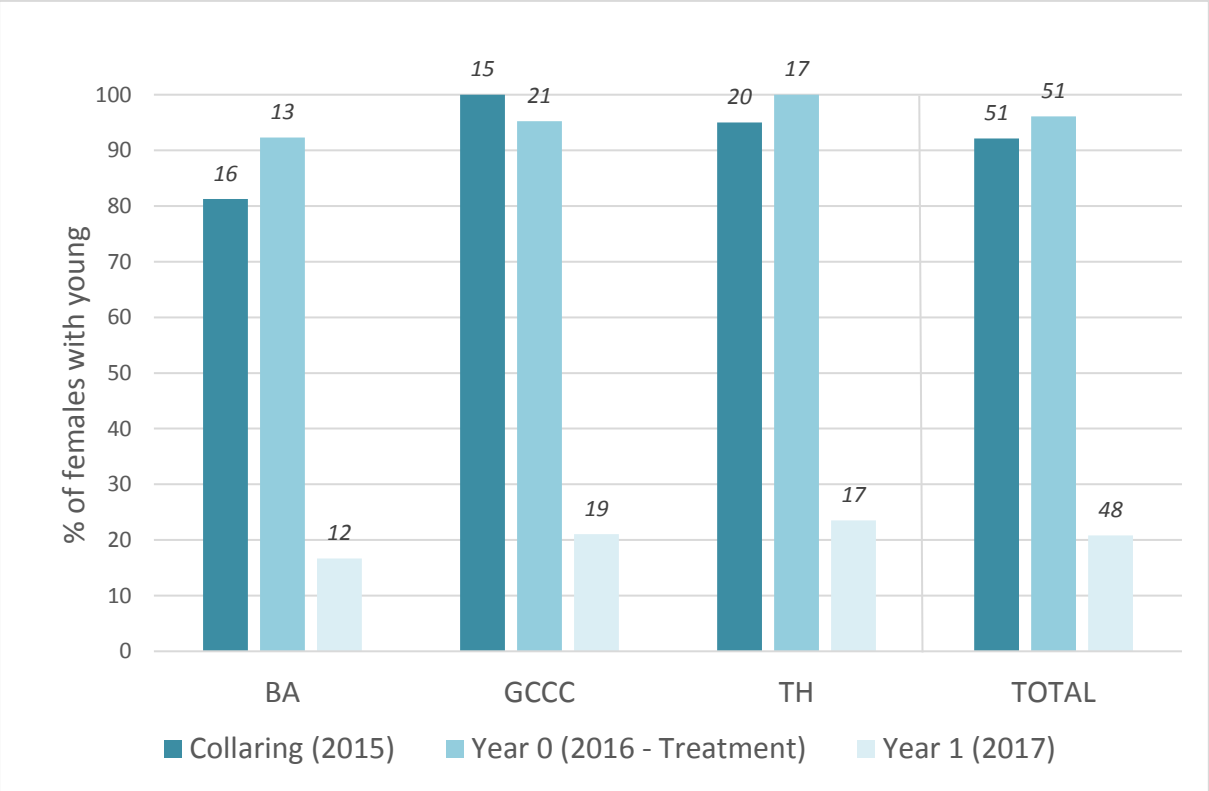


Figure 12. Percentage of female EGKs with young at the time of collaring (pre-treatment), treatment and following treatment with dart delivered GonaCon. The total number of EGKs assessed each year is presented above bars. Data for the second year post treatment is not yet available for dart administered GonaCon. **(BA = Broadcast Australia, GCCC = Gold Creek Country Club, TH = Tuggeranong Homestead, Total = all sites combined).**



3.2.5 ANNUAL POPULATION GROWTH

Population estimates were undertaken at five treated and seven untreated sites in Autumn/Winter 2015, 2016 and 2017. The annual percentage growth rates for 2015-2016 and 2016-2017 at treatment sites prior to treatment and at untreated sites varied from -35.3% to +37.1%. Following treatment with GonaCon to most of the adult females in the population in 2015, the 2016 -2017 annual growth rate was -17.6% at Weston Park (compared to 10.7% prior to treatment in 2015-2016) and -20.0% at ANBG (compared to 8.7% prior to treatment in 2015-16). At the two sites, Broadcast Australia and Gold Creek Country Club, where not all female kangaroos were treated in the population (Aim 2), the 2016 – 2017 growth rate was -5.1% and 8.6% respectively. Prior to treatment, the 2015-2016 growth rate was 36.1% at Broadcast Australia and 24.5% at Gold Creek Country Club.

3.2.6 ANNUAL POPULATION LEVEL FECUNDITY

Population level fecundity was assessed annually at each site as the proportion of adult females with a young visible in their pouch (Figures 13-15). In 2015, prior to any GonaCon treatment, the population level fecundity was high at all sites, with between 0.66 and 0.91 of adult females having a pouch young.

In the year after treatment (2016), the proportion of adult females with pouch young decreased to 0.00 at ANBG and 0.18 (± 0.02 SE) at Weston Park. Fecundity at these sites was similar in the following year (2017), with 0.00 at ANBG and 0.17 (±0.02 SE) at Weston Park. At Tuggeranong Homestead fecundity decreased to 0.15 (±0.01 SE) in the year following GonaCon treatment (2017) (Figure 13). At the two sites where not all females were treated, the proportion of adult females with pouch young was 0.54 (±0.03 SE) in 2016 and 0.45 (±0.01 SE) in 2017 at Broadcast Australia and 0.64 (±0.04 SE) in 2016 and 0.41 (±0.04 SE) in 2017 at Gold Creek Country Club (Figure 14). The proportion of adult females with pouch young remained above 0.63 at all untreated sites in all years (Figure 15).

Figure 13. Population level fecundity at population level GonaCon treatment sites (Aim 3). Hash symbols indicate the year GonaCon treatment commenced. **(ANBG = Australian National Botanic Gardens, WP = Weston Park, TH = Tuggeranong Homestead).**

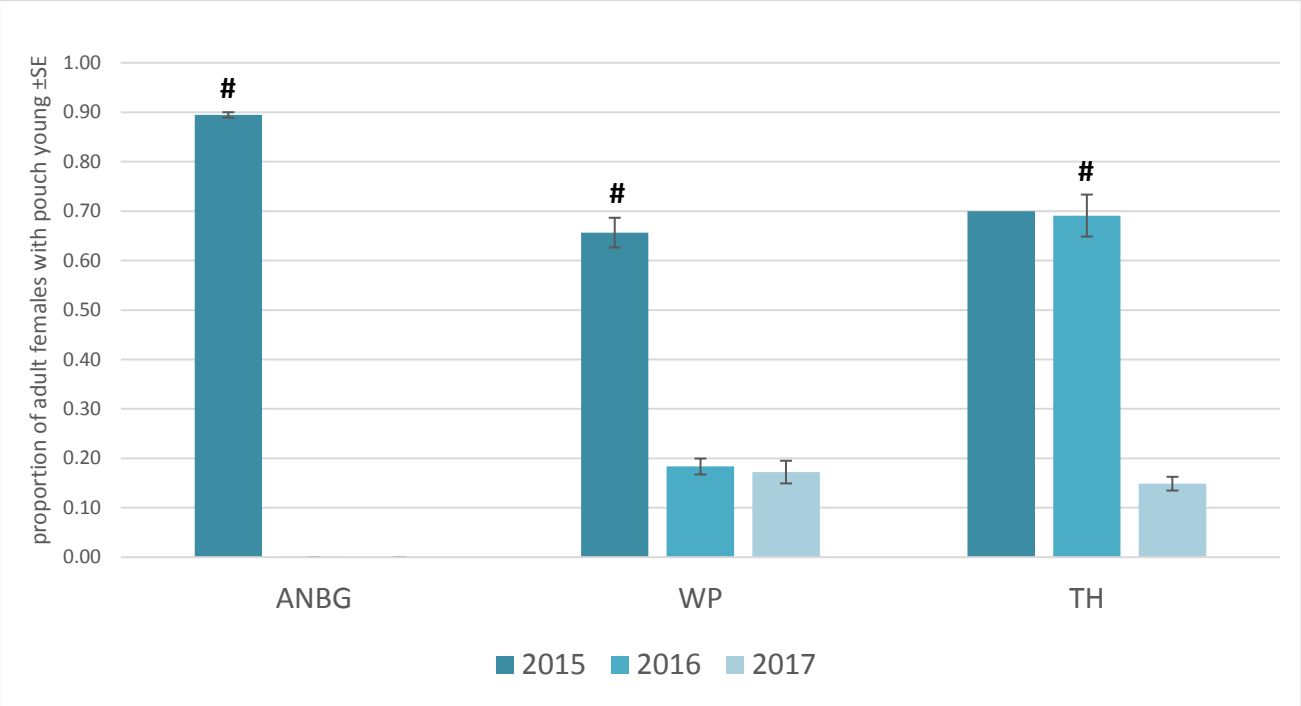


Figure 14. Population level fecundity at individual assessment sites (Aim 2). Hash symbols indicate the year GonaCon treatment commenced. (BA = Broadcast Australia, GCCC = Gold Creek Country Club).

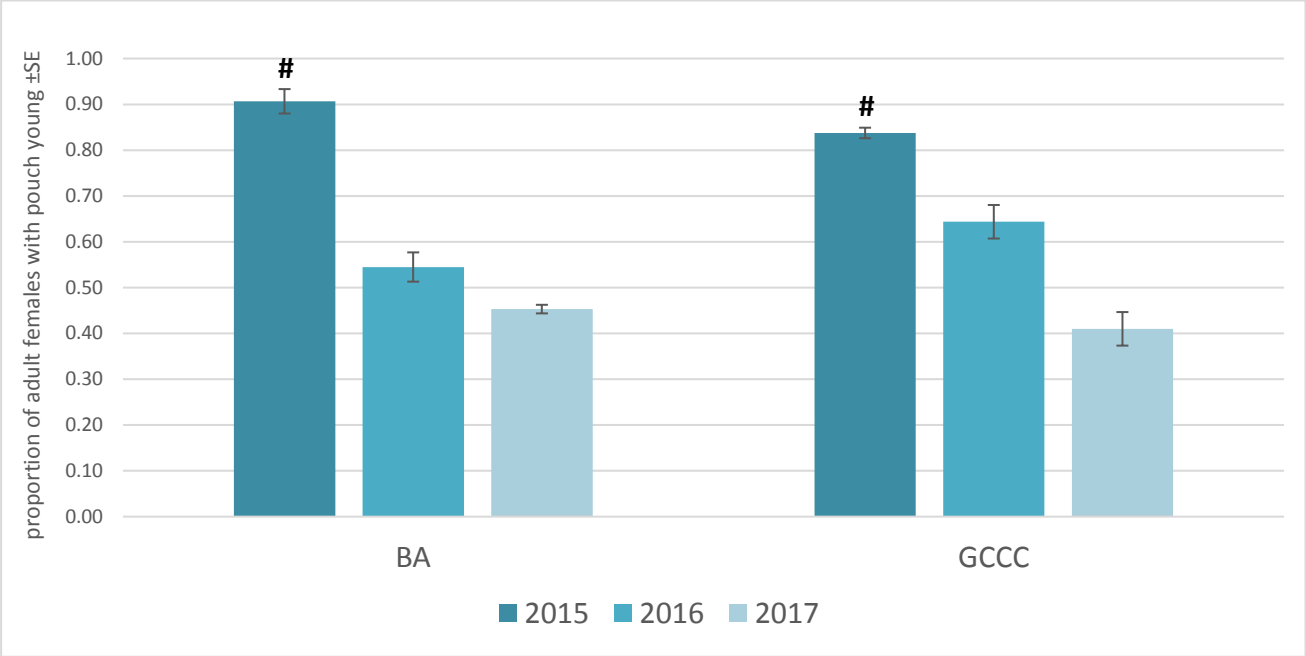
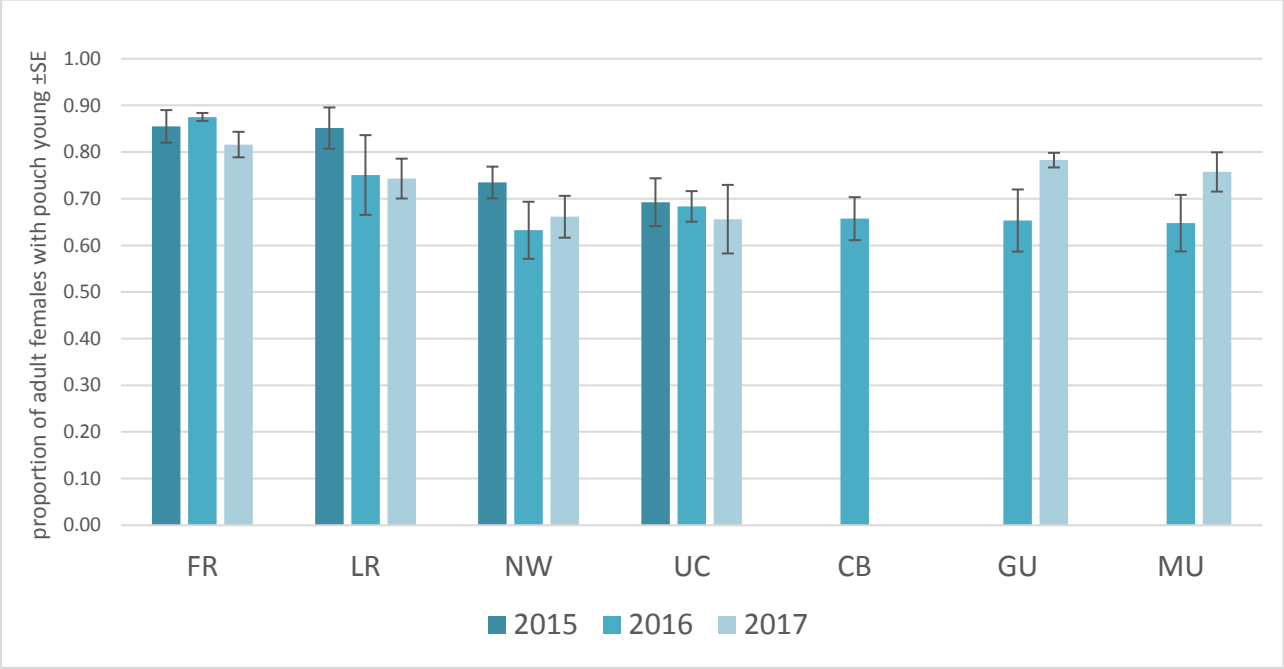


Figure 15. Population level fecundity at untreated sites (Aim 3). (FR = Farrer Ridge, LR = Lyneham Ridge/Kaleen Horse Paddocks, NW = North Weston, UC = University of Canberra, CB = Callum Brae, GU = Gungaderra, MU = Mulanggari). Note: Callum Brae was not successfully assessed in 2017 because kangaroos were too flighty.



4 DISCUSSION

4.1 DART DELIVERY OF GONACON TO FEMALE EASTERN GREY KANGAROOS

This study has identified a suitable dart system for remotely delivering GonaCon to female EGKs. As expected, dart delivery results in a more dispersed vaccine deposit, with more vaccine located subcutaneously, sub-fascially and intermuscularly, compared to injection by hand. At this stage it is unclear if the more dispersed deposit will have an impact on the long-term efficacy of the vaccine.

Based on visual assessment, the welfare impacts of administering GonaCon using a dart are comparable to darting with anaesthetic drugs for capture. In both cases, superficial bruising was present 5 days after darting but had largely resolved by 30 days. The continued presence of vaccine reactions at dart impact sites thereafter were expected, given the injection of foreign material to induce the kangaroo's immune system to produce antibodies against GnRH. No infection was identified in any of the dart sites. Some EGKs showed a very slight limp when hopping after dart vaccination but this resolved within a few days and no impairments to mobility were subsequently observed. Minor, short-term limping has also been observed in some cases after capture darting.

A temporary marking method will be essential if dart delivered GonaCon is deployed in free-ranging populations of EGKs for management purposes. The ideal delivery method would combine the GonaCon injection with the marking system in one dart. Whilst the Pneu-Dart injection-marker dart appeared appropriate, these darts are heavy and could not be accurately fired at a velocity that achieved an acceptable impact force. The alternative two-shooter method, where separate GonaCon and marker darts were fired simultaneously, had mixed success. In the 2016 trial, 42% of marker darts missed the target EGK. This was due to lack of coordination of the shooters (where the marker dart firer acted in response to the GonaCon firer's cues), and did improve with practice and experience. The GonaCon and marker darts also have different weights and trajectories. It was particularly difficult to coordinate the impact of the two darts at distances over 20-25m.

The two-shooter method is likely to be an acceptable option for small populations of EGKs that are habituated to people and easy to dart from less than 25m. In these sites, there is a high chance that any individuals that are not successfully marked on the first shot can be followed and subsequently marked before moving on to the next animal. The method is not expected to be effective in larger populations and where darting distances are greater. Further investigation of smaller, lighter injection-marker darts or other integrated marking systems is required before dart delivered GonaCon could be deployed in a management scenario. The refined system needs to include design features that widen the area of marker paint spray onto the fur, allowing treated animals to be identified more quickly and easily.

4.2 EFFICACY OF HAND INJECTED AND DART DELIVERED GONACON

The efficacy of GonaCon was high in the year following treatment with only 13.3% of hand injected and 20.8% of dart delivered female EGKs producing a young. In previous trials of GonaCon in sub-adult EGKs, none of the treated animals produced a young in the year following treatment (Snape 2012). The lower rate of infertility in the first year of this study is likely due to the timing of vaccination. At the time of treatment, one of the hand injected EGKs that subsequently bred was supporting a small young-at-foot and the remaining seven individuals had large pouch young of an average age of 247 days. It is possible that the immune response in these individuals had not generated sufficient antibodies to induce fertility before they were ready to breed again.

Alternatively, these adult females may have been carrying quiescent blastocysts prior to treatment (observed in females with young over 160 days of age; Poole and Catling 1974) and these reactivated to produce a subsequent young. Each of these theories is supported by the fact that these individuals have not bred subsequently. Similarly, all but one of the dart delivered EGKs that produced a young following treatment was observed to have medium or large pouch young when the vaccine was administered. The rate of post-treatment births is expected to be lowest if GonaCon is deployed in winter, when breeding and births are least likely to occur and sufficient time exists for an antibody response prior to the spring/summer breeding season.

At this stage it is unclear if there is any difference between the long term efficacy of dart delivered and hand injected GonaCon. It is expected, however, that some dart delivery attempts that appear to be successful will fail to inject the vaccine. A vaccine deposit could not be located in one of the four dart delivered EGKs that have been killed in motor vehicle collisions. Opportunistic data will continue to be collected from any dart delivered EGKs that die through misadventure to estimate the injection failure rate.

While the early efficacy results are positive, continued monitoring of the treated EGKs is essential to fully evaluate and compare the two GonaCon delivery systems and determine if long-lasting infertility is achievable with a single dose of dart delivered GonaCon.

4.3 LIMITING THE RATE OF INCREASE OF EASTERN GREY KANGAROO POPULATIONS USING GONACON

The high population level fecundity rates recorded prior to GonaCon treatment and in all untreated sites are consistent with estimates made at other sites in the ACT region (Fletcher 2006). Population level administration of GonaCon decreased the fecundity in all three treated populations in the year following treatment. At the two sites where treatment commenced in 2015 (ANBG and Weston Park), fecundity remained low for a second year. Only small numbers of new breeding females (0-5) were identified and dart vaccinated at the three population level sites in subsequent years. This indicates that most, if not all, breeding females were successfully treated in the first year and immigration of females into these populations was zero or very low.

As might be expected, these short term results obtained after only two years do not show a clear effect of GonaCon treatment on annual population growth. Both of the population level treatment sites where treatment commenced in 2015 (ANBG and Weston Park) show negative annual growth rates for the 2016-2017 period, which is the first year an effect would be expected. However, some of the untreated sites also had negative growth rates. Such variation is not unexpected; annual growth rates of between -2% and +40% have been measured in sites across the ACT in recent years (ACT Government 2016) with variations relating to either site-specific or environmental influences. This natural fluctuation indicates that long term trends in treated and untreated populations must be used to assess the effect of GonaCon administration to a high proportion of females on EGK population growth.

At Broadcast Australia and Gold Creek Country Club, the administration of GonaCon (to assess individual efficacy as per Aim 2) to less than 25% of female EGKs in the populations decreased fecundity to an intermediate level following the hand injected treatments in 2015. Fecundity at these sites decreased further following additional treatments via dart delivery in 2016, at which time the percentage of females treated at these sites had increased to between 30% and 50%. The population growth rates also decreased for these sites following treatment. Ongoing monitoring results from

these sites will be extremely useful for future modelling to predict the minimum proportion of animals that need to be rendered infertile to achieve the desired level of population control. Based on research and modelling in other species, it is predicted that a high proportion of female EGKs will need to be treated to suppress the growth rate and maintain population control in the longer term (Bomford and O'Brien 1992, Barlow et al 1997; Hone 1992; 2004).

4.4 MANAGEMENT IMPLICATIONS

The high efficacy of GonaCon and the decrease in fecundity observed so far in this project indicate that, if these results can be maintained, dart delivered GonaCon could be a successful fertility control option for free ranging populations of EGKs. However, the effect on population growth is not yet clear. Further monitoring of the treated animals and populations is required to determine if an acceptable level and duration of population control can be achieved with this method.

The dart delivery method developed in this study is more efficient than capture and injection by hand. The capture, hand injection treatment and care during processing and recovery for a single kangaroo is estimated to take a minimum of 4-5 hours. For efficient darting and processing of multiple animals a team of 4-6 people is desirable. For dart vaccination, only two dart gun shooters and a driver/observer are required and, depending on how easily the kangaroos can be approached, treatment of an individual can take as little as 10 minutes from locating the animal to recovering the dart. It is important to acknowledge however that time taken to locate and dart a suitable target is highly dependent on the behaviour of the animals and how accustomed they are to the presence of vehicles and people. Also, the time taken for each individual will increase as the number of individuals left to be treated decreases. Dart recovery (critical at these sites due to the risk of dart-stick injury to the public) usually took less than 10 minutes, but on a small number of occasions when the dart stayed in the kangaroo for a prolonged period of time or the kangaroo moved a large distance after being darted, it took several hours to recover the dart. Regardless, dart delivery was 50% more efficient than dart capture and subsequent hand-injection at Tuggeranong Homestead, where it took five days to complete the initial capture and collaring of 17 animals, but only 2.5 days to later dart vaccinate these individuals.

The population management approach adopted in this study at the three population level assessment sites (Aim 3 – ANBG, Tuggeranong Homestead, Weston Park) was to vaccinate as many adult females as possible in the first year (although, three sub-adult females were also treated at these sites) and then only treat adult females with pouch young in subsequent years. This approach was adopted for several reasons. Firstly, it eliminated the need for individuals to be permanently marked and instead, only a temporary marker was needed to identify vaccinated individuals within a treatment period which may be 3-4 weeks in duration. Each following year only the obviously fertile animals with pouch young would be treated, whilst adult females observed without pouch young would be presumed to have been vaccinated previously. Secondly, it eliminates the difficultly of distinguishing non-breeding or sub-adult females from small males. Finally, it allows a low level of breeding in the population because each female will have at least one young before she is vaccinated. This low level of breeding is expected to balance natural mortality rates and be important in achieving the desired population size.

In addition to continuing this study, it is necessary to trial dart delivered GonaCon in some of the small ACT nature reserves where kangaroo culling is currently undertaken. This will provide an opportunity to directly assess if the use of GonaCon could reduce the amount and/or frequency of

shooting required in future years. Trialling GonaCon in this more realistic management scenario will inform modelling of the proportion of adult females that require treatment to achieve management goals and also provide more accurate estimates of the efficiency and cost effectiveness of dart delivery compared to other management techniques.

ACKNOWLEDGEMENTS

We thank Steve Henry, Richard Barnsley, Melissa Snape, Don Fletcher, Katherine Jenkins, Sam Ried, Ryl Parker, Renee Brawata, Timothy Portas, Alice Kenney and Doug Eckery for their contribution to the design, planning and delivery of this project. We also thank the many casual contractors and volunteers who undertook the kangaroo population counts and the research site managers for their support and assistance.

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