

Long-term effects of immunocontraception on wild boar fertility, physiology and behaviour

Giovanna Massei^{A,D}, Dave P. Cowan^A, Julia Coats^A, Fiona Bellamy^A, Roger Quay^A, Stéphane Pietravalle^A, Matthew Brash^B and Lowell A. Miller^C



^AFood and Environment Research Agency, Sand Hutton, York, YO26 5 LE, UK.

^BARKVETS, Givendale House, Givendale, Pocklington, YO42 1TT, UK.

^CUSDA APHIS National Wildlife Research Center, 4101 Laporte Avenue, Fort Collins, CO 80521, USA.

^DCorresponding author. Email: giovanna.massei@fera.gsi.gov.uk

Abstract

Context. Fertility control appears as a publicly acceptable alternative to lethal methods for limiting population growth in wildlife. Recently developed single-dose immunocontraceptive vaccines have induced infertility in several mammals. However, the potential side-effects and the long-term effectiveness of these contraceptives have been poorly investigated.

Aims. We tested the long-term effectiveness and potential side-effects of the single-dose gonadotrophin-releasing hormone (GnRH) vaccine GonaCon™ on captive female wild boar.

Methods. We carried out two sequential trials: Trial 1 ($n = 6$ GonaCon™-treated and 6 control wild boar) and Trial 2 which started two years later and replicated Trial 1. We assessed the effectiveness of GonaCon™ to cause infertility by measuring GnRH antibody titres, by monitoring the oestrous cycle through the concentration of faecal progesterone and by recording the sows' reproductive output in the 4–6 years following treatment. We evaluated the potential side-effects by monitoring behaviour, bodyweight and haematological and biochemical variables.

Key results. GnRH-antibody titres decreased with time but were still detectable in all females six years after vaccination with a single dose of GonaCon™. In Trial 1 none of the treated females gave birth in the six years after vaccination. In Trial 2, progesterone indicated that two of the six treated females were cycling. One of the cycling treated females gave birth one year after vaccination; the other five, including the second cycling sow, did not reproduce in the four years following vaccination. We found no differences in bodyweight, haematology, biochemistry and behaviour and no obvious sign of injection site reaction.

Conclusions. GonaCon™ can suppress reproduction in wild boar with no long-term effects on behaviour and physiology. Therefore, GonaCon™ can be regarded as an effective and safe contraceptive for this species.

Implications. The lack of evidence of adverse effects and the longevity of effect of GonaCon™ suggest that this contraceptive could be now tested in field trials and in contexts where culling of overabundant populations of wild boar is unfeasible, illegal or unacceptable. These instances include urban areas, parks, and management of diseases where culling might cause social perturbation and result in increased disease transmission rates.

Received 29 November 2011, accepted 13 April 2012, published online 28 May 2012

Introduction

Current trends of landscape development and human population growth suggest that human–wildlife conflicts, often associated with overabundant or expanding animal populations, are likely to increase in the near future (Messmer 2009; White and Ward 2010). Traditional methods employed to mitigate these conflicts, such as culling or toxicants, can be inefficient in the long term, environmentally hazardous and may compromise animal welfare (Waddell *et al.* 2001; Delsink *et al.* 2006; Massei *et al.* 2010a). At the same time, growing public antipathy towards lethal control places increasing constraints on management options, particularly for species that have a high public profile (Barr *et al.* 2002; Deigert *et al.* 2003; Thornton and Quinn 2009). Translocation of problem animals is often advocated as a

humane alternative to culling. However, translocations are expensive, may have negative effects on animal welfare, can be responsible for introduction of pathogens and may not provide long-term solutions (Massei *et al.* 2010a). Conversely, fertility control has the potential to offer a long-term, effective and humane means of reducing the size and growth of overabundant wildlife populations (Fagerstone *et al.* 2002; Rutberg and Naugle 2008). Increasing numbers of theoretical and empirical models on the effects of fertility control on population dynamics of wildlife suggest that this approach could be as effective as or even more effective than culling to reduce population size (Hobbs *et al.* 2000; Merrill *et al.* 2003; Bradford and Hobbs 2008). Theoretical models also predict that fertility control could play a significant role in disease

management when used alongside vaccination: for instance, immunocontraception added to rabies vaccination could improve rabies control campaigns by reducing the proportion of the population that must be treated, or by reducing the duration of the vaccination campaign (Smith and Cheeseman 2002; Ramsey 2007; Carroll *et al.* 2010; Massei *in press*).

A contraceptive suitable for field applications in wildlife management (Fagerstone *et al.* 2010; Gionfriddo *et al.* 2011) should: (1) render a high proportion of animals infertile for several years after administration of a single dose, (2) have nil or minimal negative side-effects, (3) target preferentially females but ideally be also effective on males, (4) be relatively inexpensive, and (5) be available as an oral formulation. Recently developed, single-dose immunocontraceptives meet most of these criteria, although these compounds are currently available only as injectable formulations.

Immunocontraceptive vaccines act by inducing antibodies against proteins or hormones essential for reproduction (Delves 2002). The gonadotropin-releasing hormone (GnRH) peptide used in GnRH immunocontraceptives is secreted by the hypothalamus of the brain and stimulates the secretion of luteinising and follicle-stimulating hormones by the anterior pituitary gland (Miller *et al.* 2008a). These hormones, in turn, activate hormone and gamete production by the ovary and by the testis. GnRH-based immunocontraceptives cause the production of GnRH antibodies, thus preventing the production of sex hormones and ultimately inhibiting ovulation and spermatogenesis. Several GnRH-based immunocontraceptive vaccines have been developed and tested on many species of mammals, including humans. Most of these vaccines, designed for livestock and companion animals, are administered in multiple doses (reviewed in McLaughlin and Aitken 2010) and, as such, they are unsuitable for wildlife applications. However, single-dose GnRH-based injectable vaccines appear more promising for wildlife management (Fagerstone *et al.* 2002; Kirkpatrick *et al.* 2011). Among these, the single-dose injectable GnRH vaccine GonaCon™ was found to cause infertility for 1–5 years in many species of mammals (e.g. Miller *et al.* 2000, 2008b; Killian *et al.* 2008a; Massei *et al.* 2008; Gray *et al.* 2010).

Few studies have investigated the duration of induced infertility in conjunction with the potential long-term side-effects of GonaCon™ on physiology and behaviour. A study carried out by Massei *et al.* (2008) on captive female wild boar established that GonaCon™ had rendered all treated sows ($n=6$) infertile for at least one year and that in the four months following vaccination GonaCon™ had had no effect on the behaviour and on the haematological and biochemical variables of the sows ($n=12$ treated and 12 controls), although treated females had greater bodyweight than controls. However, if fertility control is going to be used to manage populations of long-lived animals, such as wild boar and other ungulates, the potential side-effects and long-term effectiveness of a contraceptive must be assessed. Evaluating the long-term effectiveness of a contraceptive is needed to predict the effects of fertility control, in terms of proportion of infertile animals and longevity of effect, on population size. For territorially and hierarchically structured species, contraception-induced changes

in social behaviour, such as decreased aggressiveness, could lead to disruption of social hierarchies and spacing behaviour and, in turn, affect the impact of fertility control on a population (Saunders *et al.* 2002; Crawford *et al.* 2011). The potential impact of a contraceptive on social behaviour should thus be evaluated before fertility control is used to manage wildlife populations.

This study expanded the work initiated by Massei *et al.* (2008) and aimed at evaluating the long-term effectiveness and potential side-effects of GonaCon™ on physiology and behaviour of captive female wild boar. Wild boar was used as a model species because of its worldwide distribution, as native or introduced species, and its impact on human interests. The latter include damage to crops and livestock, spread of diseases, vehicle collisions and reduction in plant and animal abundance and richness (e.g. Hone 2002; Engeman *et al.* 2004; Massei and Genov 2004; Conover 2007). Although the species can be controlled through hunting (Massei *et al.* 2011), it is essential to evaluate different options to manage overabundant populations, particularly in urban and/or protected areas where culling is unfeasible or undesirable.

Materials and methods

The study was conducted in two trials, Trial 1 and Trial 2, between 2004 and 2010.

In 2004, 2-year-old wild boar females ($n=12$) of proven fertility were obtained from a local farm and housed as a single group in three interconnected outdoor paddocks (each 77×24 m). Animals were fed on commercial pig diet, offered *ad libitum* water and equipped with coloured ear-tags for individual identification. Six females were unexpectedly found to be pregnant and births occurred in early July. All the piglets were removed by a veterinarian within 3 days of birth. On 17 August 2004 females were randomly assigned to Treatment group ($n=6$) and injected intramuscularly in the rump with 1000 µg of GonaCon, or to Control group ($n=6$) and injected with the adjuvant only. Each group comprised 3 previously pregnant and 3 non-pregnant females. Trial 2 was designed to replicate Trial 1 and to monitor the effects of GonaCon™ on the reproductive cycle of wild boar. On 26 April 2006 new female wild boar were randomly assigned to the Treatment ($n=6$) or Control group ($n=6$), as in Trial 1. Controls in Trial 2 were injected with a saline solution only as Trial 1 had shown that the adjuvant had no obvious adverse injection site reaction (Massei *et al.* 2008). In both trials, the vaccine contained the mollusc protein Keyhole limpet hemocyanin as a carrier.

Two males were introduced with the sows of Trial 1 in November 2004. Once births had occurred, following the introduction of the males into the paddocks, all control females and the piglets born in Trial 1 were removed from the experiment in August 2005. Therefore, from April 2006, the following groups of wild boar were studied: Treated in Trial 1, Treated in Trial 2 and Control in Trial 2. All treated females from Trial 1 were housed continuously with a male wild boar from May 2006 till December 2010.

In June 2007 one male was introduced for three months with the sows of Trial 2. After births had occurred, all piglets were removed. In January 2010 the male boar normally housed with

the females in Trial 1 was introduced again for three months with the sows of Trial 2.

Effectiveness of GonaCon™ to induce long-term infertility

The effectiveness of the vaccine to maintain infertility was determined by collecting the following data: (1) immune response to the vaccine, assessed by measuring serum antibodies to the GnRH, (2) concentration of faecal progesterone used as an indicator of cycling, and (3) reproductive output.

Blood samples were collected from all sows under anaesthesia at vaccination, 6 and 12 weeks after vaccination and thereafter every 6–9 months. Animals were anaesthetised by a mixture of Zoletil® (tiletamine–zolazepam), Zalopine® (medetomidine hydrochloride) and Torbugesic® (butorphanol tartrate) administered by dart gun. The enzyme-linked immunosorbent assay (ELISA) was used to measure anti-GnRH antibody titres. Fifty microlitres of wild boar serum were used for each assay. A 96-well plate was prepared by adding 100 ng of Bovine Serum Albumin (BSA)-GnRH antigen to each well and then blocking with SeaBlock from Pierce Chemical. Antibodies to GnRH in the wild boar serum were detected by placing a BSA-GnRH on the ELISA plate. Wild boar serum was serially diluted from 1 : 1000 to 1 : 128 000 in phosphate-buffered saline containing SeaBlock. Antibodies in the wild boar serum to GnRH on the plate were detected with the following linkages: rabbit anti-wild boar IgG, bound to the wild boar IgG, and horseradish peroxidase (HRP)-goat anti-rabbit bound to the rabbit IgG. Chromogen tetramethylbenzidine was used to develop the colour, and 2 M H₂SO₄ was used to stop the reaction. The colour intensity of the sample was read at 450 nm with a Dynatech MR 5000 ELISA plate and expressed as an absorbance value. Anti-GnRH antibody titres were calculated by comparing blood samples between pre- and postvaccination, and expressed as the highest dilution (i.e. 1:16 000, 1:32 000 or 1:64 000) in which the postvaccination sample had a higher absorbance value than that of the prevaccination sample.

Samples with titres higher than 1 : 128 000 were rerun with serial dilution from 1 : 4000 to 1 : 512 000.

During the wild boar breeding season, between 23 November and 17 December 2009, faecal samples were collected by staff who followed individual animals until the faecal sample was produced. Samples were collected twice a week from all females of Trial 2 and then processed as described in Massei *et al.* (2008). A fully validated ELISA was used to measure the concentration of progesterone as described in Massei *et al.* (2008).

Reproductive output was assessed by the number of females giving birth. Differences in the proportions of treated and control animals pregnant at the end of the study, in 2010, were analysed by Fisher's Exact Test. Bodyweight was recorded every 6–9 months in parallel with blood collection for antibody titres.

Effects of GonaCon™ on behaviour and physiology

To determine whether vaccination with GonaCon™ affected the social rank of wild boar, behavioural data on agonistic interactions with trial mates were collected as described in

Massei *et al.* (2008) and compared between trials and before and after vaccination. In Trial 1 prevaccination was in July–August 2004 and postvaccination in August 2004–March 2005; in Trial 2 prevaccination was in November 2005–April 2006 and postvaccination was in April 2006–April 2007. In brief, the behaviour of each animal was observed in 3-h sessions carried out once or twice every fortnight and the identity of the animal initiating or receiving an agonistic interaction was noted. The Barrette and Vandal (1986) index on agonistic interactions (AI) was derived for each animal as follows:

$$\text{AI index} = (\text{no. of AI initiated} + 1) / (\text{no. of AI received} + 1)$$

A REML analysis was carried out on the AI indices to test for the effect of trial, treatment (treated versus control) and vaccination period (before and after vaccination).

Differences in bodyweight between groups were analysed by an Analysis of Covariance (ANCOVA). Data were log₁₀-transformed and the initial weight, recorded at vaccination, was used as covariate.

A general blood chemistry panel was performed on blood samples by Carmichael Torrance Diagnostic Services Ltd (Leeds, UK). For each sample, the following biochemical parameters were collected: α-, β- and gamma-globulins, ionised calcium, albumin, urea, creatinine, alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase, sodium, potassium, calcium and bile acids. The following haematological parameters were also collected: haemoglobin, packed cell volume, red blood cell count, white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin concentration, neutrophils and lymphocytes. Data from the last collection date were used to compare biochemical and haematological values of treated and control females. In October 2009, the following animals were sampled: four treated sows in Trial 1, 62 months after vaccination, and 12 sows in Trial 2 (*n* = 6 treated and 6 controls) 42 months after vaccination. Differences in haematological and biochemical values between treated and control animals were analysed with a Multivariate Analysis of Variance (MANOVA). Data analyses were carried out in GENSTAT 13 (Payne 2003).

The study was carried out under a UK Home Office licence, in accordance with the *Animals (Scientific Procedures) Act 1986* and was approved by the Food and Environment Research Agency's Ethical Review Process.

Results

Effectiveness of GonaCon™ to induce long-term infertility

Two treated animals in Trial 1 died under anaesthesia, one in July 2007 and one in October 2009, for causes believed to be unrelated to the vaccine. The analysis of GnRH antibody titres showed a long-term, sustained immune response in both groups of treated wild boar (Fig. 1). Notably, after an initial drop in the 12–24 months following vaccination, antibody titres stayed relatively but consistently low (1 : 64 000) in subsequent years and were associated with infertility in 11 out of 12 treated animals (see below). Antibodies were not measured in control animals as these sows were not expected to have anti-GnRH titres.

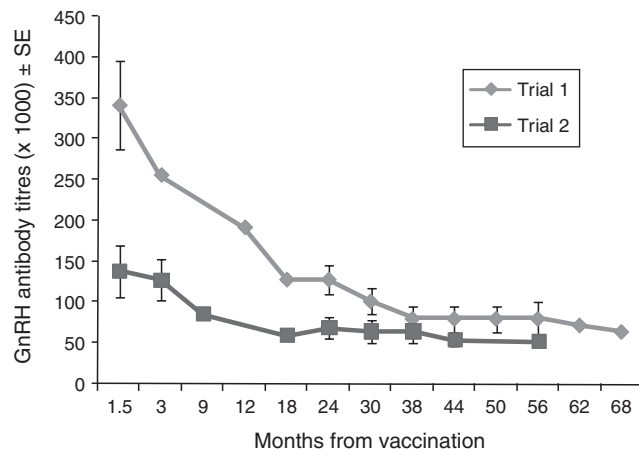


Fig. 1. Mean anti-GnRH antibody titres (±s.d.) of female wild boar treated with GonaCon™ in Trial 1 (n=6) and Trial 2 (n=6).

The results on progesterone levels indicated that in all 6 control and in 2 treated females in Trial 2 progesterone peaked from 100–500 ng g⁻¹ during the follicular phase preceding ovulation to 8000–15 000 ng g⁻¹ during the luteal phase following ovulation (Table 1). The GnRH antibody titres of the two treated animals that appeared to cycle (PuPu and RG), measured 1 : 32 000 whereas the titres of all the other wild boar were ≥ 1 : 64 000. In all the other four treated females progesterone levels varied between 67 and 1761 ng g⁻¹.

In October 2007 all control females in Trial 2 and one treated female (PuPu) gave birth. In May–June 2010, five control females in Trial 2 and the same treated female (PuPu) gave birth to 5–7 piglets per litter. The control that did not give birth (YPu) was cycling like the other controls but had dry blood around the genital area at the end of May 2010, suggesting that she might have aborted a litter. The other treated female (RG) that was cycling and had relatively low antibody titres did not give birth. In summary, in 2010, 4–6 years after treatment with GonaCon™, nine out of the 10 treated females were still infertile and at least 5 out of 6 control females gave birth. The difference in reproductive output between treated and control was significant (Fishers’s Test *P* = 0.008).

Effects of GonaCon™ on behaviour and physiology

The Agonistic Interaction index varied with treatment ($\chi^2 = 48.85$, d.f. = 1, *P* < 0.001) but not with trial ($\chi^2 = 3.56$, d.f. = 1, *P* = 0.06), vaccination period ($\chi^2 = 2.48$, d.f. = 34, *P* = 1), treatment*trial ($\chi^2 = 0.35$, d.f. = 1, *P* = 0.55) and treatment*vaccination period ($\chi^2 = 21.35$, d.f. = 34, *P* = 0.95). For ease of presentation, the AI indices were converted to social ranks (Fig. 2) derived by calculating an AI for each animal on each day and by ranking the AIs from 1 to 12, with 12 indicating the highest social rank. The results of the analysis indicated that animals in treated groups had initially higher AI indices than controls but that vaccination had no effect on aggressiveness as both groups of animals maintained their ranks throughout the trials.

Bodyweight increased with time in both groups. In Trial 2, the bodyweight of treated and control females did not differ ($F_{1,59} < 1.0$, *P* = 0.665) up to 54 months after vaccination (Fig. 3).

There were no differences between GonaCon™-treated and control wild boar in biochemical (Wilk’s statistic = 0.0896, $F_{14,1} = 0.726$, *P* = 0.740) or haematological (Wilk’s statistic = 0.8722, $F_{9,6} = 0.098$, *P* = 0.999) variables (Table 2). No signs of lameness were noticed in treated animals in the years following vaccination and no obvious granulomata or abscesses were recorded at the injection site when animals were examined under anaesthesia by a veterinarian during regular health checks.

Discussion

Our study showed that a single dose of GonaCon™ induced infertility for at least 3–6 years in 11 out of 12 female wild boar. This contraceptive prevented treated animals from cycling but did not affect their physiology and behaviour, thus confirming earlier results (Massei *et al.* 2008). Treatment with GonaCon™ did not affect aggressiveness; the difference between trials was borderline significant (*P* = 0.06) and might have been due to small sample size. In addition, the temporary bodyweight increase in treated females observed three months after vaccination (Massei *et al.* 2008) did not persist: at the end of this study, four years after vaccination, the bodyweight of treated animals in Trial 2 did not differ from that of controls. The results on progesterone levels indicated that all six control and two treated females in Trial 2 were cycling although only one of these treated females gave birth. This was consistent with other studies; for instance, white-tailed deer treated with a

Table 1. Faecal progesterone concentration (in ng g⁻¹) recorded between November and December 2009 in individual wild boar females in Trial 2. Treated sows were vaccinated in April 2006. Shaded cells indicate animals that were cycling, as demonstrated by very high concentration of faecal progesterone (highlighted in bold for each sow)

	Control						Treated					
	GG	PiW	PuY	RY	WO	YPu	GB	GPu	PiPi	PuO	PuPu	RG
23 November	3823	11 825	7560	284	4725	8579	287	385	146	149	4700	8683
26 November	4212	1696	13 526	454	13 335	6988	130	83	1761	290	6223	1211
30 November	3686	8323	2535	8980	12 814	3409	179	200	258	171	1737	814
3 December	8199	426	1139	128	205	609	213	375	494	222		
8 December	471	2075	194		759	249	155	240	70	212	3882	14 909
10 December	1470	1759	140	101	6748	365	977	143	68	784	8792	716
14 December	5519	1141	2944	3069	6998	2427	146	197	67	178	1060	4549
17 December	5887	2897	6840	617	7401	1717	234	168	81	96	10 031	9569

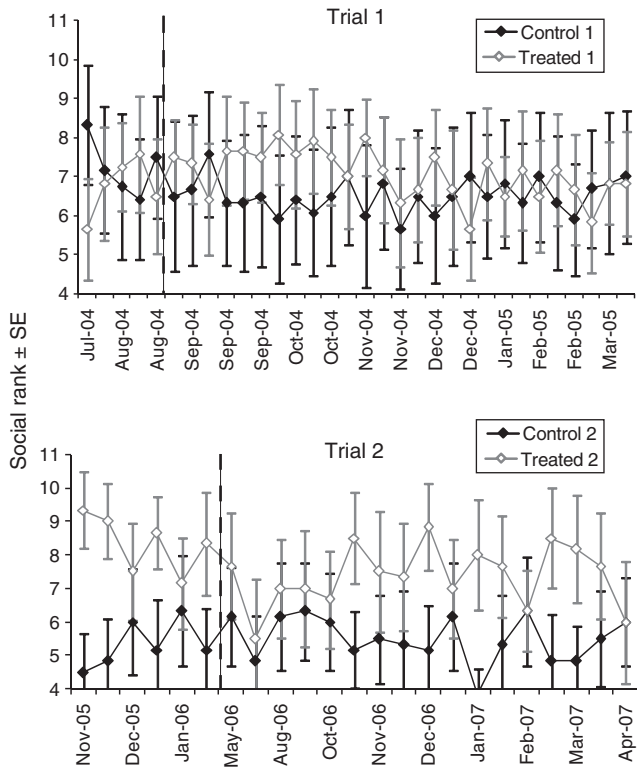


Fig. 2. Mean social rank of treated and control wild boar females in Trial 1 and Trial 2 before and after vaccination with GonaCon™. Vaccination date is indicated by a dotted line. The social rank was derived from the Agonistic Interaction index (AI)=(no. of wins+1)/(no. of losses+1); AIs were calculated for each animal on each day and ranked so that they ranged from 1 to 12, with 12 indicating the highest social rank.

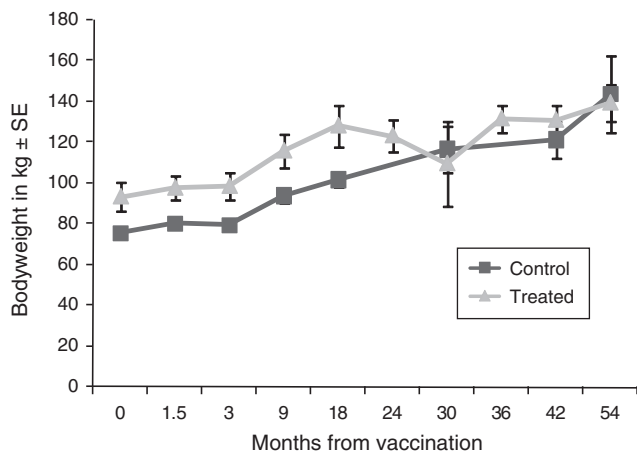


Fig. 3. Mean bodyweight (±s.d.) of control ($n=6$) and GonaCon™-treated ($n=6$) wild boar females in Trial 2.

single-shot Porcine Zona Pellucida immunocontraceptive that showed signs of breeding behaviour and increased progesterone levels indicating oestrous did not become pregnant in the following season (Miller et al. 2009).

Table 2. Mean haematological and biochemical variables (±s.d.) recorded in October 2010 for wild boar females (control and treated with a single dose of the immunocontraceptive GonaCon™ in 2004 and 2006)

Variable	Control ($n=6$)	Treated ($n=10$)
Albumin (g dL ⁻¹)	33.24 ± 2.78	33.74 ± 2.21
Alpha-globulins (g dL ⁻¹)	0.90 ± 1.06	12.13 ± 1.60
Beta-globulins (g dL ⁻¹)	1.92 ± 0.79	13.28 ± 2.28
Gamma-globulins (g dL ⁻¹)	14.10 ± 2.03	13.94 ± 2.24
Ionised calcium (mmol L ⁻¹)	1.20 ± 0.06	1.19 ± 0.09
Urea (mmol L ⁻¹)	4.1 ± 0.90	4.42 ± 0.81
Creatinine (µmol L ⁻¹)	172.70 ± 70.40	170.20 ± 22.89
Alkaline phosphatase (IU L ⁻¹)	24.33 ± 4.18	18.70 ± 6.70
Aspartate aminotransferase (IU L ⁻¹)	51.17 ± 10.94	48.00 ± 15.30
Gamma-glutamyl transferase (IU L ⁻¹)	55.00 ± 9.65	43.50 ± 7.41
Sodium (mmol L ⁻¹)	143.33 ± 3.93	142.10 ± 2.13
Potassium (mmol L ⁻¹)	4.82 ± 0.52	4.99 ± 0.77
Calcium (mmol L ⁻¹)	2.24 ± 0.08	2.28 ± 0.10
Bile acids (µmol L ⁻¹)	1.12 ± 1.03	0.87 ± 0.64
Haemoglobin (g dL ⁻¹)	12.28 ± 1.51	12.39 ± 0.88
Packed cell volume (%)	0.37 ± 0.04	0.36 ± 0.03
Red blood cell count (10 ¹² L ⁻¹)	5.37 ± 0.59	5.36 ± 0.42
White blood cell count (10 ⁹ L ⁻¹)	4.87 ± 01.05	4.97 ± 1.77
Mean corpuscular volume (fl)	68.17 ± 1.94	67.7 ± 2.16
Mean corpuscular haemoglobin concentration (g dL ⁻¹)	33.45 ± 0.26	34.09 ± 1.68
Mean corpuscular haemoglobin (pg)	22.87 ± 0.65	23.13 ± 1.25
Neutrophils (10 ⁹ L ⁻¹)	2.82 ± 1.05	2.60 ± 1.71
Lymphocytes (10 ⁹ L ⁻¹)	1.73 ± 0.49	2.09 ± 0.87

Similarly, in deer treated with GonaCon™ the reproductive behaviour occurred 1–2 years before the animals became fertile again (Killian et al. 2008b). A possible explanation is that follicular development and the production of oestrogen are sufficient to support reproductive behaviour but inadequate to restore fertility (Killian et al. 2008b).

This study tested simultaneously the effectiveness and the potential side-effects of a contraceptive on wild boar over 4–6 years. Most studies carried out on single-dose immunocontraceptives in wildlife focussed either on effectiveness (e.g. Miller et al. 2008b; Killian et al. 2008a; Gray et al. 2010) or on potential side-effects (e.g. Kirkpatrick and Turner 2007; Curtis et al. 2008; Gionfriddo et al. 2011; Yoder and Miller 2011). Results from other studies indicate that the long-term effectiveness of GonaCon™, in terms of both duration of infertility and proportion of animals rendered infertile, varies in relation to species and possibly health condition of the animals. For instance, a single injection of GonaCon™ administered to females rendered 100% ($n=6$) of bison (*Bison bison*) and feral pigs ($n=9$) infertile for at least a year (Miller et al. 2004; Killian et al. 2006a), 91% of California ground squirrels (*Spermophilus beecheyi*) infertile in Year 1 ($n=33$) and Year 2 ($n=24$) after vaccination (Nash et al. 2004) and 100% ($n=10$) of elk (*Cervus elaphus*) infertile for 3 years (Killian et al. 2009). Long-term studies showed that GnRH antibody titres decrease with time and that reproduction may resume in some animals in the years following vaccination. Some of these studies suggested that

the effectiveness of GonaCon™, measured as the proportion of individuals rendered infertile, was greater in captivity than in field trials. For instance GonaCon™ caused infertility in 80% ($n = 5$) of captive white-tailed deer (*Odocoileus virginianus*) for five years (Miller *et al.* 2008b) but a subsequent study carried out with free-living animals (Gionfriddo *et al.* 2009) reported infertility in 88% and 47% of the deer ($n = 28$) during the first and second year after vaccination. Similarly, in captive wild horses GonaCon™ caused infertility in 94%, 60% and 53% of the mares ($n = 15$) in Year 1, 2 and 3 following vaccination (Killian *et al.* 2008a) but in free-roaming feral horses GonaCon™ caused infertility in 39%, 42% and 31% of the mares (Gray *et al.* 2010). These differences might be due to the possibility that the better body conditions, usually found in captive animals, might affect immunocompetence and hence the strength and persistence of the immune response to the vaccine (Gray *et al.* 2010). Differences in immune response to vaccines might also be due to species, age, sex, reproductive status (pregnant versus non-pregnant animals), genetic variation in populations and exposure to other antigens (e.g. Buehler *et al.* 2011; Demas *et al.* 2011). Understanding the role these factors play in shaping an individual's response to immunocontraception will assist to optimise fertility control applications in terms of candidate species, timing of treatment and context. For instance, if pregnancy were found to affect immunocompetence, animals could be treated before the reproductive peak; likewise, animals might be vaccinated outside seasonal food shortages that might affect their health and thus their immune response. Field trials with free-living wild boar should be conducted to determine whether the results found in this study can be replicated in field conditions.

Several studies found no side-effects of GonaCon™ on the physiology and behaviour of animals treated with GonaCon™. For instance, GonaCon™ did not affect bodyweight or blood chemistry of black-tailed prairie dogs (*Cynomys ludovicianus*) (Yoder and Miller 2011) and blood chemistry in white-tailed deer (Killian *et al.* 2006b). However, other studies in white-tailed deer, cats and elk reported that animals treated with GonaCon™ developed palpable non-painful injection site granulomata or sterile abscesses that did not seem to affect the welfare of the animals (Killian *et al.* 2006b; Curtis *et al.* 2008; Gionfriddo *et al.* 2009; Levy *et al.* 2011). The granuloma at the injection site plays a prime role in the host's defence against the 'chronic infection' represented by a multiyear immunocontraceptive and is considered necessary to the efficacy of the adjuvant (Miller *et al.* 2008a). As all methods employed in wildlife management to decrease population size have advantages and disadvantages (e.g. Massei *et al.* 2011), the occurrence and potential implications of side-effects must be viewed against alternative options to contraception such as lethal control.

The results of this study confirmed that injectable GonaCon™ met the safety and effectiveness criteria required for an ideal fertility control agent. For wildlife applications, an oral immunocontraceptive would be preferable to an injectable one as it would eliminate the costs of trapping and handling animals. However, as GnRH is widely conserved among mammals, it is likely that an oral formulation could affect non-target species (Levy 2011). Thus an oral GnRH vaccine would require the use of a species-specific delivery system, such as those developed

to deliver pharmaceuticals to wild pigs (Long *et al.* 2010; Massei *et al.* 2010b).

In the current injectable formulation, immunocontraceptives can have a variety of field applications in situations where culling is not feasible, desirable or legal. These include control of overabundant wildlife in urban areas and parks, iconic species for which culling is publicly unacceptable, and protected areas where hunting is not allowed. Other applications of these immunocontraceptives concern instances when population size reduction is required for disease control, particularly when alternative options, such as culling, have the potential for social perturbation. Several authors highlighted that culling can affect social behaviour and lead to increased disease transmission whilst fertility control is unlikely to have similar effects (e.g. Smith and Cheeseman 2002; McDonald *et al.* 2008; Killian *et al.* 2009; Harrison *et al.* 2010). In addition, fertility control can induce behavioural changes, such as reproduction-related long-distance movements, that reduce disease transmission rates (e.g. Caley and Ramsey 2001; Ramsey *et al.* 2002; Ramsey 2007).

GonaCon™ is currently registered in the US as a contraceptive for white-tailed deer (Fagerstone *et al.* 2010). Whilst many traditional forms of intervention are subject to increasing public criticism, the longevity, safety and social acceptability of this immunocontraceptive for managing overabundant wildlife offer a credible alternative to lethal control. Field trials are now needed to test the feasibility, effectiveness and costs of managing free-living populations through fertility control based on immunocontraception.

Acknowledgements

This study was funded by the Department for Environment, Food and Rural Affairs under projects WM0406 and WM0408. The authors are grateful to the technical staff at the Food and Environment Research Agency's Animal Unit for their help throughout the project and to the members of the Ethical Review Panel. Thanks to Kathy Fagerstone and Richard Brand-Hardy for reading and commenting on the first draft.

References

- Barr, J. J. F., Lurz, P. W. W., Shirley, M. D. F., and Rushton, S. P. (2002). Evaluation of immunocontraception as a publicly acceptable form of vertebrate pest species control: the introduced grey squirrel in Britain as an example. *Environmental Management* **30**, 342–351. doi:10.1007/s00267-002-2686-7
- Barrette, C., and Vandal, D. (1986). Social rank dominance, antler size and access to food in snow-bound wild woodland caribou *Rangifer tarandus caribou*. *Behaviour* **97**, 118–145. doi:10.1163/156853986X00342
- Bradford, J. B., and Hobbs, N. T. (2008). Regulating overabundant ungulate populations: an example for elk in Rocky Mountain National Park, Colorado. *Journal of Environmental Management* **86**, 520–528. doi:10.1016/j.jenvman.2006.12.005
- Buehler, D. M., Versteegh, M. A., Matson, K. D., and Tieleman, B. I. (2011). One problem, many solutions: simple statistical approaches help unravel the complexity of the immune system in an ecological context. *PLoS ONE* **64**, e185992.
- Caley, P., and Ramsey, D. (2001). Estimating disease transmission in wildlife, with emphasis on leptospirosis and bovine tuberculosis in possums, and effects of fertility control. *Journal of Applied Ecology* **38**, 1362–1370. doi:10.1046/j.0021-8901.2001.00676.x

- Carroll, M. J., Singer, A., Smith, G. C., Cowan, D. P., and Massei, G. (2010). The use of immunosuppression to improve rabies eradication in urban dog populations. *Wildlife Research* **37**, 676–687. doi:10.1071/WR10027
- Conover, M. R. (2007). America's first feral hog war. *Human-Wildlife Conflicts* **1**, 129–131.
- Crawford, J., Boulet, M., and Drea, C. M. (2011). Smelling wrong: hormonal contraception in lemurs alters critical female odour cues. *Proceedings. Biological Sciences* **278**, 122–130. doi:10.1098/rspb.2010.1203
- Curtis, P. D., Richmond, M. E., Miller, L. A., and Quimby, F. W. (2008). Physiological effects of gonadotropin-releasing hormone immunosuppression on white-tailed deer. *Human-Wildlife Conflicts* **2**, 68–79.
- Deigert, F. A., Duncan, A. E., Frank, K. M., Lyda, R. O., and Kirkpatrick, J. F. (2003). Immunosuppression of captive exotic species. III. Contraception and population management of fallow deer (*Cervus dama*). *Zoo Biology* **22**, 261–268. doi:10.1002/zoo.10081
- Delsink, A. K., Van Altena, J. J., Grobler, D., Bertschinger, H., Kirkpatrick, J. F., and Slotow, R. (2006). Regulation of a small discrete African elephant population through immunosuppression in the Makalali Conservancy, Limpopo, South Africa. *South African Journal of Science* **102**, 403–405.
- Delves, P. J. (2002). Antifertility vaccines. *Trends in Immunology* **23**, 213–219. doi:10.1016/S1471-4906(02)02182-8
- Demas, G. E., Zysling, D. A., Beechler, B. R., Muehlenbein, M. P., and French, S. S. (2011). Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology* **80**, 710–730. doi:10.1111/j.1365-2656.2011.01813.x
- Engeman, R. M., Smith, H. T., Severson, R., Severson, M. A., Woolard, J., Shwift, S., Constantin, B. U., and Griffin, D. (2004). Damage reduction estimates and benefit–cost ratios for feral swine control from the last remnant of a basin marsh system in Florida. *Environmental Conservation* **31**, 207–211. doi:10.1017/S0376892904001572
- Fagerstone, K. A., Coffey, M. A., Curtis, P. D., Dolbeer, R. A., Killian, G. J., Miller L. A., and Wilmot, L. M. (2002). Wildlife fertility control. The Wildlife Society Technical Review 02–2.
- Fagerstone, K. A., Miller, L. A., Killian, G. J., and Yoder, C. A. (2010). Review of issues concerning the use of reproductive inhibitors, with particular emphasis on resolving human–wildlife conflicts in North America. *Integrative Zoology* **5**, 15–30. doi:10.1111/j.1749-4877.2010.00185.x
- Gionfriddo, J. P., Eisemann, J. D., Sullivan, K. J., Healey, R. S., Miller, L. A., Fagerstone, K. A., Engeman, R. M., and Yoder, C. A. (2009). Field test of a single-injection gonadotropin-releasing hormone immunosuppressive vaccine in female white-tailed deer. *Wildlife Research* **36**, 177–184. doi:10.1071/WR08061
- Gionfriddo, J. P., Denicola, A. J., Miller, L. A., and Fagerstone, K. A. (2011). Health effects of GnRH immunosuppression of wild white-tailed deer in New Jersey. *Wildlife Society Bulletin* **35**, 149–160. doi:10.1002/wsb.17
- Gray, M. E., Thain, D. S., Cameron, E. Z., and Miller, L. A. (2010). Multi-year fertility reduction in free-roaming feral horses with single-injection immunosuppressive formulations. *Wildlife Research* **37**, 475–481. doi:10.1071/WR09175
- Harrison, A., Newey, S., Gilbert, L., Haydon, D. T., and Thirgood, S. (2010). Culling wildlife hosts to control disease: mountain hares, red grouse and louping ill virus. *Journal of Applied Ecology* **47**, 926–930. doi:10.1111/j.1365-2664.2010.01834.x
- Hobbs, N. T., Bowden, D. C., and Baker, D. L. (2000). Effects of fertility control on populations of ungulates: general, stage-structured models. *Journal of Wildlife Management* **64**, 473–491. doi:10.2307/3803245
- Hone, J. (2002). Feral hogs in Namadgi National Park, Australia: dynamics, impacts and management. *Biological Conservation* **105**, 231–242. doi:10.1016/S0006-3207(01)00185-9
- Killian, G. J., Miller, L. A., Rhyan, J. C., and Doten, H. (2006a). Immunosuppression of Florida feral swine with a single-dose GnRH vaccine. *American Journal of Reproductive Immunology* **55**, 378–384. doi:10.1111/j.1600-0897.2006.00379.x
- Killian, G., Eisemann, J. D., Wagner, D., and Werner, J., Shaw, D., Engeman, R., and Miller, L. A. (2006b). Safety and toxicity evaluation of GonaCon immunosuppressive vaccine in white-tailed deer. In 'Proceedings of the 22nd Vertebrate Pest Conference'. (Eds R. M. Timm and J. M. O'Brien.) pp. 82–87. (University of California: Davis, CA.)
- Killian, G., Thain, D. S., Diehl, N., Rhyan, J., and Miller, L. (2008a). Four-year contraception rates of mares treated with single-injection porcine zona pellucida and GnRH vaccines and intrauterine devices. *Wildlife Research* **35**, 531–539. doi:10.1071/WR07134
- Killian, G., Wagner, D. C., Fagerstone, K., and Miller, L. (2008b). Long-term efficacy and reproductive behaviour associated with GonaCon™ use in white-tailed deer (*Odocoileus virginianus*). In 'Proceedings of the 23rd Vertebrate Pest Conference'. (Eds R. M. Timm and M. B. Madon.) pp. 240–243. (University of California: Davis, CA.)
- Killian, G., Kreeger, T. J., Rhyan, J., Fagerstone, K., and Miller, L. (2009). Observations on the use of GonaCon™ in captive female elk (*Cervus elaphus*). *Journal of Wildlife Diseases* **45**, 184–188.
- Kirkpatrick, J. F., and Turner, A. (2007). Immunosuppression and increased longevity in equids. *Zoo Biology* **26**, 237–244. doi:10.1002/zoo.20109
- Kirkpatrick, J. F., Lyda, R. O., and Frank, K. M. (2011). Contraceptive vaccines for wildlife: a review. *American Journal of Reproductive Immunology* **66**, 40–50. doi:10.1111/j.1600-0897.2011.01003.x
- Levy, J. K. (2011). Contraceptive vaccines for the humane control of community cat populations. *American Journal of Reproductive Immunology* **66**, 63–70. doi:10.1111/j.1600-0897.2011.01005.x
- Levy, J. K., Friary, J. A., Miller, L. A., Crawford, P. C., Tucker, S. J., and Fagerstone, K. A. (2011). Long-term fertility control in female cats with GonaCon™, a GnRH immunosuppressive. *Theriogenology* **76**, 1517–1525. doi:10.1016/j.theriogenology.2011.06.022
- Long, D. B., Campbell, T. A., and Massei, G. (2010). Evaluation of feral swine-specific feeder systems. *Rangelands* **32**, 8–13. doi:10.2111/1551-501X-32.2.8
- Massei, G. (in press). Fertility control in dogs. In 'Dogs, Zoonoses and Public Health'. (Eds C. N. L. Macpherson, F. X. Meslin and A. I. Wandeler.) (CABI Publishing: Oxford.)
- Massei, G., and Genov, P. (2004). The environmental impact of wild boar. *Galemys* **16**, 135–145.
- Massei, G., Cowan, D. P., Coats, J., Gladwell, F., Lane, J. E., and Miller, L. A. (2008). Effect of the GnRH vaccine GonaCon™ on the fertility, physiology and behaviour of wild boar. *Wildlife Research* **35**, 540–547. doi:10.1071/WR07132
- Massei, G., Quay, R., Gurney, J., and Cowan, D. P. (2010a). Can translocations be used to manage human–wildlife conflicts? *Wildlife Research* **37**, 428–439. doi:10.1071/WR08179
- Massei, G., Coats, J., Quay, R., Storer, K., and Cowan, D. P. (2010b). The BOS (Boar-Operated-System): a novel method to deliver baits to wild boar. *Journal of Wildlife Management* **74**, 333–336. doi:10.2193/2008-489
- Massei, G., Roy, S., and Bunting, R. (2011). Too many hogs? A review of methods to mitigate impact by wild boar and feral pigs. *Human-Wildlife Interactions* **5**, 79–99.
- McDonald, R. A., Delahay, R. J., Carter, S. P., Smith, G. C., and Cheeseman, C. L. (2008). Perturbing implications of wildlife ecology for disease control. *Trends in Ecology & Evolution* **23**, 53–56. doi:10.1016/j.tree.2007.10.011
- McLaughlin, E. A., and Aitken, R. J. (2010). Is there a role for immunosuppression? *Molecular and Cellular Endocrinology*. ePub.
- Merrill, J. A., Cooch, E. G., and Curtis, P. D. (2003). Time to reduction: factors influencing management efficacy in sterilizing overabundant white-tailed deer. *Journal of Wildlife Management* **67**, 267–279. doi:10.2307/3802768

- Messmer, T. A. (2009). Human–wildlife conflicts: emerging challenges and opportunities. *Human–Wildlife Conflicts* **3**, 10–17.
- Miller, L. A., Johns, B. E., and Killian, G. J. (2000). Immunocontraception of white-tailed deer with GnRH vaccine. *American Journal of Reproductive Immunology* **44**, 266–274. doi:10.1111/j.8755-8920.2000.440503.x
- Miller, L. A., Rhyan, J. C., and Drew, M. (2004). Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. *Journal of Wildlife Diseases* **40**, 725–730.
- Miller, L. A., Fagerstone, K. A., Kemp, J., and Killian, G. J. (2008a). Immune mechanisms and characterization of injection site reactions involved in the multi-year contraceptive effect of the GonaCon™ vaccine. In 'Proceedings of the 23rd Vertebrate Pest Conference'. (Eds R. M. Timm and M. B. Madon.) pp. 244–249. (University of California: Davis, CA.)
- Miller, L. A., Gionfriddo, J. P., Fagerstone, K. A., Rhyan, J. C., and Killian, G. J. (2008b). The single-shot GnRH immunocontraceptive vaccine (GonaCon™) in white-tailed deer: comparison of several GnRH preparations. *American Journal of Reproductive Immunology* **60**, 214–223. doi:10.1111/j.1600-0897.2008.00616.x
- Miller, L. A., Fagerstone, K. A., Wagner, D. C., and Killian, G. J. (2009). Factors contributing to the success of a single-shot, multiyear PZP immunocontraceptive vaccine for white-tailed deer. *Human–Wildlife Conflicts* **31**, 103–115.
- Nash, P. B., James, D. K., Hui, L., and Miller, L. A. (2004). Fertility control of California ground squirrels using GnRH immunocontraception. In 'Proceedings of the 21st Vertebrate Pest Conference'. (Eds R. M. Timm and W. P. Gorenzel.) pp. 274–278. (University of California: Davis, CA.)
- Payne, R. W. (2003). 'GENSTAT 7.1 Numerical Algorithms Group.' (VSN International: Oxford.)
- Ramsey, D. (2007). Effect of fertility control on behavior and disease transmission of brush-tailed possum. *Journal of Wildlife Management* **71**, 109–116. doi:10.2193/2005-699
- Ramsey, D., Spencer, N., Caley, P., Efford, M., Hansen, K., Lam, M., and Cooper, D. (2002). The effect of population density on contact rates between male and female brushtail possums *Trichosurus vulpecula* and the implications for transmission of bovine tuberculosis. *Journal of Applied Ecology* **39**, 806–818. doi:10.1046/j.1365-2664.2002.00760.x
- Rutberg, A. T., and Naugle, R. E. (2008). Population-level effects of immunocontraception in white-tailed deer. *Wildlife Research* **35**, 494–501. doi:10.1071/WR07128
- Saunders, G., McIlroy, J., Berghout, M., Kay, B., Gifford, E., Perry, R., and Van De Ven, R. (2002). The effects of induced sterility on the territorial behaviour and survival of foxes. *Journal of Applied Ecology* **39**, 56–66. doi:10.1046/j.1365-2664.2002.00696.x
- Smith, G. C., and Cheeseman, C. L. (2002). The control of diseases in wildlife populations: culling, vaccine and fertility control. *Ecological Modelling* **150**, 45–53. doi:10.1016/S0304-3800(01)00471-9
- Thornton, C., and Quinn, M. S. (2009). Coexisting with cougars: public perceptions, attitudes, and awareness of cougars on the urban–rural fringe of Calgary, Alberta, Canada. *Human–Wildlife Conflicts* **3**, 10–17.
- Waddell, R. B., Osborn, D. A., Warren, R. J., Griffin, J. C., and Kesler, D. J. (2001). Prostaglandin F-2 alpha-mediated fertility control in captive white-tailed deer. *Wildlife Society Bulletin* **29**, 1067–1074.
- White, P. C. L., and Ward, A. I. (2010). Interdisciplinary approaches for the management of existing and emerging human–wildlife conflicts. *Wildlife Research* **37**, 623–629. doi:10.1071/WR10191
- Yoder, C. A., and Miller, L. A. (2011). Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. *Vaccine* **29**, 233–239. doi:10.1016/j.vaccine.2010.10.055

