

Final Project Report

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 Research Policy and International Division, Final Reports Unit
 DEFRA, Area 301
 Cromwell House, Dean Stanley Street, London, SW1P 3JH.
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Project title	Evaluation of fertility control in captive wild boar		
DEFRA project code	WM0306		
Contractor organisation and location	Central Science Laboratory Sand Hutton York YO41 1LZ		
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Executive summary (maximum 2 sides A4)

1. Defra seeks to promote safe, effective and humane means of resolving conflicts between wildlife and human interests. Traditional methods of wildlife management are often ineffective in the long-term, environmentally hazardous, publicly unacceptable and uneconomic. The development of more benign methods is thus vital for the future of effective and socially acceptable wildlife management.
2. Fertility control has the potential to be used as an alternative to lethal methods for limiting population growth in overabundant species. The most promising fertility control agents are immunocontraceptive vaccines which stimulate the immune system to produce antibodies that neutralise proteins essential for reproduction. Among these agents, the newly developed Gonadotropin Releasing Hormone (GnRH) vaccine suppresses the activity of GnRH which, in turn, interrupts the hormonal processes leading to ovulation and sperm production. Through the addition of novel adjuvants to boost the immune response, single-dose GnRH vaccines have been demonstrated to induce infertility experimentally in a variety of mammal species. Such “single-shot” vaccines represent a major technological breakthrough that makes some practical applications realistic. However, the potential side effects of these vaccines on animal behaviour, physiology and welfare remain poorly understood.
3. The present study focussed on the wild boar as a model species in which to investigate the effectiveness and potential side effects of the GnRH vaccine on the physiology, behaviour, and welfare of individual animals. Wild boar have recently colonised several areas in the UK, due to escapes from farms and have considerable potential for negative impacts on rural and conservation

interests. It is thus essential to evaluate different options for managing the possible expansion, both in terms of distribution and local densities, of wild boar populations in the UK.

4. Twelve captive wild boar females were housed in outdoor paddocks, ear tagged and assigned to treatment and control groups. Vaccination was carried out three months later: treated sows were injected with the GnRH vaccine (n = 6) and controls with the adjuvant only (n=6). Males were introduced to the paddocks 13 weeks after vaccination. The effectiveness of the vaccine for inducing infertility was determined by measuring serum antibodies to the GnRH vaccine, by using the concentration of faecal progesterone as an indicator of oestrus cycling, pregnancy and maintenance of pregnancy, and by determining the reproductive output of both groups. Behavioural data on time budgets and dominance were collected in the periods before and after-vaccination. Physiological data were derived from health profiles based on blood serum samples collected at vaccination and 6 and 12 weeks after vaccination. Body weight was also measured at these times. Cortisol was used as an indicator of stress and its concentration was determined in faecal samples collected before and after vaccination.
5. Six control females and none of the treated females gave birth. Progesterone levels increased in both treated and control females in mid September, suggesting the onset of an oestrus cycle. The levels of progesterone in the treated females fell and remained low from week 12 onwards with no cycling apparent. Very high levels of progesterone were uniquely associated with pregnancy. No differences between the time budgets of treated and control females were observed during the pre- and post-vaccination periods. In autumn, both groups spent progressively more time feeding and walking and less time sleeping than in summer. No differences between treated and control females were observed in the total numbers of agonistic interactions initiated and received and in social ranks. No differences in blood parameters were observed between groups. Over time, body weight increase was more pronounced in treated females than in control females. The levels of cortisol did not differ in treated and control groups during the post-vaccination period but increased in pregnant females.
6. The results of this study showed that the GnRH vaccine significantly reduces the reproductive output of wild boar. With the exception of an increase in body weight for treated females, no other significant side effects of the vaccine were observed on the behaviour, physiology and welfare of the animals. This indicated that, at least in the short-term, the GnRH vaccine can be regarded as an effective, humane and safe method to induce infertility in individual wild boar.
7. Wildlife management is a particularly sensitive area in which opinions tend to be polarised. Against this challenging background effective fertility control methods would provide Defra with (i) the possibility of advocating non-lethal methods for managing overabundant populations and (ii) enhanced objective decision making about best practice for wildlife management. However, in order to build on this project's very encouraging proof of concept using emerging technology in captive animals, studies of free-living animals are required to confirm the long-term effectiveness and humaneness of the technique. Confirmation in such studies, of the promise reported here for this model species, would offer real prospects of practical applications being realised for fertility control, with the potential to revolutionise our approach to wildlife management in the UK.

Scientific report (maximum 20 sides A4)**1. Introduction**

Traditional attempts to resolve conflicts between wildlife and human interests are frequently ineffective, environmentally hazardous, uneconomic and compromise animal welfare (e.g. Waddell *et al.* 2001, Fagerstone *et al.* 2002, Smith & Cheeseman 2002). At the same time, growing public antipathy towards lethal methods places increasing constraints on management options, particularly for those species that have a high public profile (Jackson 2001, Barr *et al.* 2002, Deigert *et al.* 2003). Humane, sustainable, environmentally sensitive, non-lethal approaches to managing overabundant or expanding populations are thus increasingly required. Few such approaches are currently available and some, such as live-capture and translocation, cannot be regarded as long-term solutions. However, fertility control has as yet unrealised potential to offer benign, long-term, effective and humane approaches to reducing overabundant wildlife populations (Barlow 2000, Fagerstone *et al.* 2002, Turner *et al.* 2002).

Immunocontraceptive vaccines offer great promise for fertility control in some mammalian species (Fagerstone *et al.* 2002). Immunocontraception is achieved by exposing an animal to a foreign substance (antigen) that stimulates the animal's immune system to produce antibodies. These antibodies neutralise proteins or hormones essential for reproduction (Delves *et al.* 2002). Once exposed to the vaccine, an animal will usually retain a complement of antibodies to deal with subsequent exposure. This is the principle underlying many vaccines developed to protect against diseases.

Early formulations of fertility control agents had to be delivered as a primer shot followed by a booster. This was a major constraint on practical application with wildlife, as individual animals would have to be recaptured in order to deliver the booster vaccination. These vaccine formulations also used Freund's adjuvant (FCA). An adjuvant is a compound added to the vaccine to increase the immune response of an individual to that vaccine. Some constituents of Freund's adjuvant raised concerns about the safety of this material. However, significant progress has been made by the National Wildlife Research Centre (NWRC) of the United States Department of Agriculture in the development of single-dose vaccines, capable of inducing long-term infertility, using adjuvants that do not pose risks to treated animals or potential consumers (Miller *et al.* 1999, Miller *et al.* 2004). These "single-shot" vaccines represent a major technological breakthrough that makes some practical fertility control applications realistic. Amongst these vaccines, the Gonadotropin Releasing Hormone (GnRH) vaccine offers the best prospects for fertility control in a variety of mammalian species (Miller *et al.* 2003, Miller *et al.* 2004, Killian *et al.* 2003).

The GnRH vaccine interferes with the reproductive physiology of both sexes by causing the development of antibodies that suppress the gonadotropin releasing hormone (GnRH). The GnRH, secreted from the hypothalamus in the brain, controls the release of the pituitary reproductive hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH) which in turn control the functions of ovaries and testes (Miller & Fagerstone 2000, Killian & Miller 2000). In females, the suppression of the GnRH stops ovulation, the oestrous cycle, and reduces the production of oestrogen and progesterone from the ovaries. In males, suppressing GnRH decreases the production of sperm and testosterone in the testes. The GnRH vaccine is successful in reducing fertility in many mammal species including domestic and feral pigs (Killian & Miller 2000, Fagerstone *et al.* 2002, Miller *et al.* 2003.). However, the potential side effects of the GnRH vaccine on the behaviour, physiology and welfare of treated individuals have so far received little attention.

The present study focussed on investigation, in the wild boar (*Sus scrofa*) as a model species, of the effectiveness and potential side effects of the GnRH vaccine. Wild boar have recently colonised several areas in the UK, due to escapes from farms (Goulding 2002). This species is considered to have significant potential negative impacts on rural and conservation interests. Due to a high reproductive rate coupled with an ability to adapt to a wide range of environmental conditions, wild boar can reach very high local densities (Jeziarski 1977, Massei *et al.* 1996, Massei *et al.* 1997). This often results in crop damage, decreases in plant and animal diversity, potential for disease transmission and risks of vehicle collision (Singer *et al.* 1984, Hone 1995, Artois *et al.* 2002). It is thus essential to evaluate different options to manage the possible expansion (both in terms of distribution and local densities) of wild boar populations in the UK.

The aims of project WM0306 were the following:

1. Establish proof of concept for the effectiveness of a single dose of GnRH vaccine to induce infertility in captive wild boar.
2. Assess the potential side effects of the GnRH vaccine on the physiology, behaviour, and welfare of individual wild boar.
3. Propose a programme of further research that will allow the GnRH vaccine to be tested on free-living wild boar in order to provide an effective, humane and environmentally sensitive strategy for resolving wild boar conflicts with human interests.

2. Methods

2.1. Study animals and plan of work

Twelve wild boar females were obtained from a local farm, as two separate groups of six females each, in late April 2004. All the females were housed together in three interconnected outdoor paddocks (77 x 24 m) at the CSL's Animal Care Facility. All the sows were 2 years-old and had already given birth to at least one litter. Animals were fed on commercial pig diet (Pigbreed Classic Nut Diet, BOCM Pauls Ltd., Selby Yorks.) and had *ad libitum* water. Between 20th of May and 10th June all females were anaesthetised, ear tagged and equipped with Passive Integrated Transponder (PIT) tags. Blood samples were also collected for later analyses. In late June six sows were unexpectedly found to be pregnant and births occurred between the 1st and 21st of July. All the piglets were removed by a veterinarian within 3 days of birth. On the 16th and 17th of August six sows (Treatment group) were injected intramuscularly with 1000 μ g of KLH-GnRH vaccine supplied by NWRC and six (Control group) with the adjuvant only. Each group comprised 3 previously pregnant and 3 non-pregnant females. All sows were anaesthetised again 6 and 12 weeks after vaccination (Table 1).

Table 1. Plan of work on evaluation of fertility control in captive wild boar.

Date	
29-Apr-04	Arrival 12 sows
20 May-10 June	1st anaesthesia and tagging
1-21 July 04	Births (6 sows)
16-17 August 04	2nd anaesthesia + vaccination
28-29 Sept 04	3rd anaesthesia, 6 weeks after vaccination
10-11 November 04	4th anaesthesia, 12 weeks after vaccination
18 November 04	2 males introduced in the paddocks
March	Births
Feecal sampling	from June, 1-2 sample /sow /week
Behaviour	3 hrs observation/ week
Physiology	ELISA on feecal progesterone and cortisol
	Health profile
	Body weight

2.2 Effectiveness of the GnRH vaccine to induce infertility in the wild boar

In mammals the oestrus cycle is divided into a *follicular* phase and a *luteal* phase. The follicular phase involves the maturation of the follicle and ends with ovulation. The luteal phase is characterised by secretion of progesterone by the *corpus luteum*. If the egg is not fertilised, the *corpus luteum* regresses, the progesterone levels drop and a new follicular phase begins. If the egg is fertilised the *corpus luteum* continues to produce progesterone for the first part of the pregnancy. Thereafter, the placenta secretes progesterone. Repeated sampling of progesterone levels can be used to assess the reproductive status (cycling, *anestrus*, or pregnancy) of a female. In wild boar and in domestic pigs the length of the oestrus cycle is 21 days (Henry 1968, Hulten *et al.* 1995). The follicular phase lasts usually 5 days and is followed by 16-day luteal phase. The oestrus patterns of sows can be established by measuring progesterone concentration.

As females housed together tend to have simultaneous cycling (i.e they all ovulate at approximately the same time), the control sows were expected to follow very similar cycling patterns whereas the sows treated with the GnRH vaccine were expected to show no cycling.

The effectiveness of the vaccine to induce infertility in wild boar was determined by collecting the following data:

1. immune response to the vaccine, assessed by measuring serum antibodies to the GnRH vaccine
2. blocking effect of the GnRH on reproduction, determined by measuring a reduction in the concentration of progesterone in faecal samples of the treated females compared to controls
3. concentration of faecal progesterone, to be used as a measure of cycling, pregnancy and maintenance of pregnancy
4. behavioural responses of boar to vaccinated and control sows, to be assessed by direct observations
5. reproductive output (litter size).

The concentration of antibody titres in serum samples collected at vaccination, and 6 and 12 weeks after vaccination was determined following the method described in Levy *et al.* (2004).

The concentration of progesterone was measured in faecal material. Fresh faecal samples were collected from each boar (within 1 hr after defecation) approximately twice per week from June 2004 to January 2005 and once fortnightly from January until the end of March 2005.

The faeces were dried at 25 °C immediately after collection and then ground finely and mixed. Sub-samples were extracted in duplicate with 80% methanol. The supernatant was removed, concentrated and stored at room temperature in airtight containers. Prior to assay, samples were reconstituted with phosphate buffered saline (PBS) and diluted with deionised water. Samples were assayed with commercially available, fully validated enzyme-linked immunosorbent assay (ELISA) kits for progesterone. Standards were used on each ELISA plate. Results were obtained using a plate-reader (Dynex and Labsystems Multiscan Ascent) with a 450 nm filter and plate reader software (Revelation 3.0 and Ascent).

A REML analysis was used to test the effects of time (date), treatment (vaccination) and time*treatment on the log-transformed concentration of progesterone. As the sampling dates were not equally spaced and collection dates were not the same for all the animals (due to the difficulty of collecting samples regularly and from all the sows) the REML was based on a Power correlation model (GenStat 7.1).

2.3. Potential effects of the GnRH vaccine on behaviour, physiology, and welfare

The potential effects of the GnRH vaccine on the behaviour of the wild boar females were assessed by collecting behavioural data during one 3-hour observation session per week. In each session, the behaviour of each animal was recorded every 10 minutes and attributed to one of the following activities: sleeping, lying, feeding, walking, standing, and “other” (wallowing, carrying sticks, defecating/urinating, sexual behaviour such as mounting). During the same three hours, all the social interactions such as agonistic encounters, sniffing genitals and mounting were also recorded whenever they occurred. Data from these sessions were allocated to the “Pre-vaccination” period (from 22nd July to 16th August) and “Post-vaccination” period (from the 18th August to 16th November).

The “post-mating” period started on 18th November when two mature males were introduced into the paddocks. In the two weeks following the introduction of the males seven 3-hour observation sessions were carried out (one every one to two days) to record interactions between the males and treated and control females. In this period, sexual activity was recorded as the number of mounting attempts (defined as one male mounting a female for < 5 seconds) and mating (defined as one male mounting a female for \geq 5 seconds) and the number of times males sniffed the genitals of the females.

A Principal Component Analysis (PCA) was used to summarise the time spent by treated and control wild boar in different activities during the Pre-and Post-vaccination periods. The scores of individual animals on the first PC were used in a Split-Plot ANOVA, which assumes uniform correlation structure over time. More complex correlation patterns, allowing for unequal correlation structure of the data were investigated using REML and comparing the deviance of different models. However as all these models did not significantly

improve the fit of the data, a Split-Plot ANOVA was used to test the effects of time (date), treatment (vaccination), time*treatment and period (Pre- and Post-vaccination) on the behaviour of the wild boar.

The social rank of each animal was obtained by the Barrette & Vandal (1986) index:

Rank = (Wins +1)/ (Losses +1).

Split-Plot ANOVAs were used to test the effects of time (date), treatment (vaccination), time*treatment and period (Pre- and Post-vaccination) on the total number of agonistic interactions initiated and received by each sow and on social rank.

Body weight and health profiles were used to determine whether the GnRH vaccine affected the physiology of the treated sows. Body weight was recorded at vaccination and 6 and 12 weeks after vaccination. Differences in body weight recorded for treated and control sows 12 weeks after vaccination were analysed by an Analysis of Covariance (ANCOVA). Data were Log-transformed and the initial weight, recorded at vaccination, was used as a covariate.

Health profiles were obtained from blood samples collected at vaccination and 6 and 12 weeks after vaccination. The following biochemical parameters were recorded: alpha-1 globulins, alpha-2 globulins, beta globulins, gamma globulins, ionised calcium, total proteins, albumin, urea, creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), sodium, potassium, calcium, bile acids and inorganic phosphate. The following haematological parameters were recorded: haemoglobin, Packed Cell Volume (PCV), Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Neutrophils, Lymphocytes and Monocytes.

Data from the biochemistry and haematology from the three dates (vaccination, 6 and 12 weeks after vaccination) were summarised by a Principal Component Analysis (PCA). The effects of date, treatment (vaccination), and date*treatment on the first Principal Component scores were tested by a REML analysis using Wald test statistics. Within each date, t-tests or Mann-Whitney tests were also carried out on individual blood parameters that were expected to differ as a result of vaccination.

The concentration of faecal cortisol, used as a measure of stress, was measured in the same samples collected for the analysis of progesterone. Samples were assayed with commercially available, fully validated enzyme-linked immunosorbent assay (ELISA) kits for cortisol.

The ELISA kits used to measure cortisol and progesterone were highly specific to the steroid hormone they were designed to measure and had minimal cross-reactivity with other steroid hormones. Validation experiments, carried out in this study with faecal samples, confirmed good linearity (involving serial dilutions of samples before assay) and recovery (i.e. the amount of total hormone present versus the amount of hormone detected by the assay).

A REML analysis was used to test the effects of time (date), treatment (vaccination) and time*treatment on the log-transformed concentration of cortisol. As the sampling dates were not equally spaced and collection dates were not the same for all the animals (due to the difficulty of collecting samples regularly and from all the sows), the REML was based on a Power correlation model (GenStat 7.1).

3. Results

3.1 Effectiveness of the GnRH vaccine to induce infertility in the wild boar

The analysis of the immune response to the GnRH vaccine showed that 6 weeks after vaccination all the treated wild boar had developed serum anti-GnRH titres. Twelve weeks after vaccination the concentration of antibody titres had not changed, indicating a sustained immune response over this time period.

In the post-vaccination period (weeks 1 to 13) progesterone levels changed with date ($Chi\text{-}sq. = 9.89$, $d.f. = 31$, $P < 0.001$) but not with treatment ($Chi\text{-}sq. = 1.91$, $d.f. = 1$, $P > 0.05$) or date* treatment ($Chi\text{-}sq. = 1.05$, $d.f. = 28$, $P > 0.05$). The effect of date was due to an increase in progesterone levels in both treated and control females between weeks 5 and 8 (Figure 1), which suggested the onset of an oestrus cycle for all the animals. The levels of progesterone in the treated females fell and remained low from week 12 onwards with

no cycling apparent. Very high levels of progesterone (i.e. > 5000ng/g), observed before vaccination and after the males had been introduced in the paddocks, were uniquely associated with pregnancy.

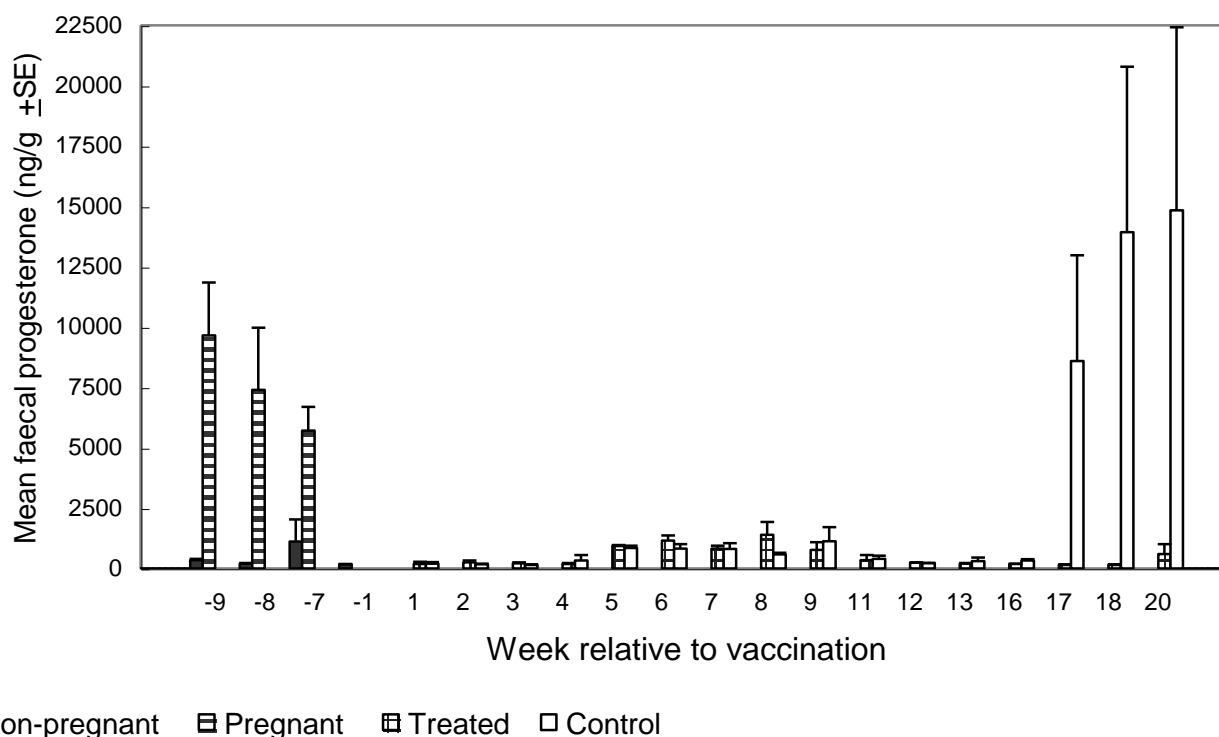


Figure 1. Mean faecal progesterone (ng/g dry faeces) for pregnant (N=6) and non-pregnant boar (N=6) before vaccination and control (N=6) and treated boar (N=6) after vaccination.

Sexual behaviour, indicated by males mounting or mating with females and/or sniffing their genitals was observed between 22nd and 26th November. No mounting was observed on 30th November and only one mounting was recorded on the 2nd December. In this period, the number of times males sniffed female genitals was 28 for control and 12 for treated females respectively. All six control females were mounted for a total number of 30 mounts. Mating was observed on 4 control females. No mating was observed on any treated sows. In the same period only one treated female (OY) was mounted 8 times by a male.

Five births occurred between the 23rd and 27th March and one birth occurred on 16th April. All six control females produced litters of 7-9 piglets each. None of the six treated females gave birth. The female that gave birth about three weeks later than the other controls had been observed mating at the same time as the others and showed high concentrations of faecal progesterone a few weeks later, indicating either pregnancy or normal cycling. Three weeks is the normal cycle length and thus she probably became pregnant on her second rather than first cycle after the males were introduced. She was observed having convulsions on two occasions after the introduction of the males. There is thus the possibility that she had a condition that interfered with her early pregnancy.

3.2. Potential effects of the GnRH vaccine on behaviour, physiology, and welfare

No limping, hunched posture or any other behavioural sign of distress were observed in either treated and control wild boar following treatment with the GnRH vaccine or with the adjuvant.

The first Principal Component, summarising the time spent by treated and control females in different activities, explained 71.3 % of the variability and contrasted “eating” with “sleeping”. The second Principal Component, explaining 21.0 % of the variability, contrasted “eating” with “walking”. Time spent in different activities was influenced by date ($F_{19,190} = 35.67$, $P < 0.001$) but not by treatment ($F_{1,10} = 0.01$, $P > 0.05$), treatment*time interaction ($F_{19,190} = 1.23$, $P > 0.05$) and vaccination period ($F_{1,208} = 0.87$, $P > 0.05$). For both

treated and control females differences in behaviour over time, as indicated by the first PC, were mainly due to a change in eating and sleeping patterns. Both groups spent progressively more time eating and less time sleeping as autumn approached (Figure 2).

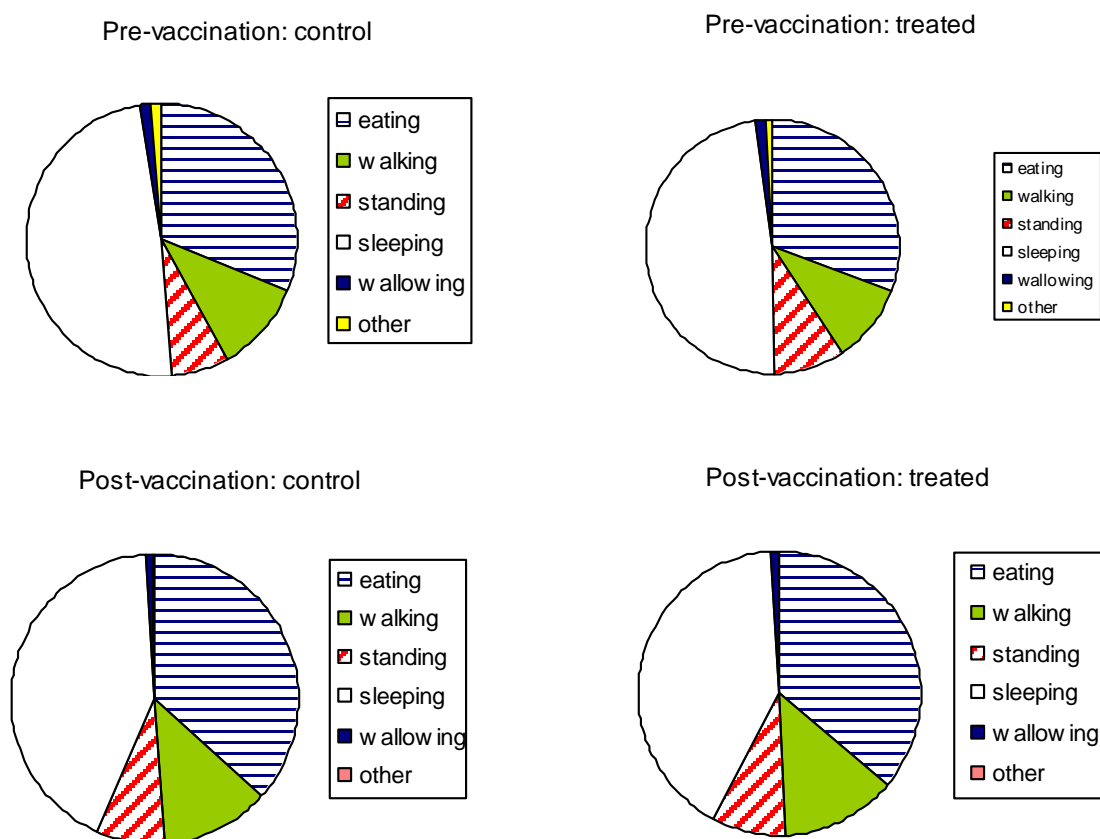


Figure 2. Time budget by control and treated wild boar groups before and after treatment with the GnRH vaccine.

The number of agonistic interactions initiated was influenced by date ($F_{19,19} = 4.87$, $P < 0.001$) but not by treatment ($F_{1,10} = 0.01$, $P > 0.05$), treatment*time interaction ($F_{19,190} = 0.96$, $P > 0.05$) or vaccination period ($F_{1,208} = 0.00$, $P > 0.05$). Similarly, the number of agonistic interactions received was influenced by date ($F_{19,19} = 6.93$, $P < 0.001$) but not by treatment ($F_{1,10} = 0.90$, $P > 0.05$), treatment*time interaction ($F_{19,190} = 1.30$, $P > 0.05$) or vaccination period ($F_{1,208} = 0.38$, $P > 0.05$) (Figure 3).

Social ranks remained stable throughout the study period and were not influenced by date ($F_{19,190} = 0.00$, $P > 0.05$), treatment ($F_{1,10} = 0.16$, $P > 0.05$), treatment*time interaction ($F_{19,190} = 1.17$, $P > 0.05$) or vaccination period ($F_{1,208} = 0.12$, $P > 0.05$) (Figure 4).

Body weight increased with time in both groups (Figure 5). Twelve weeks after vaccination the body weight of treated sows had increased more than that of controls (ANCOVA $F_{1,9} = 6.96$, $P = 0.027$).

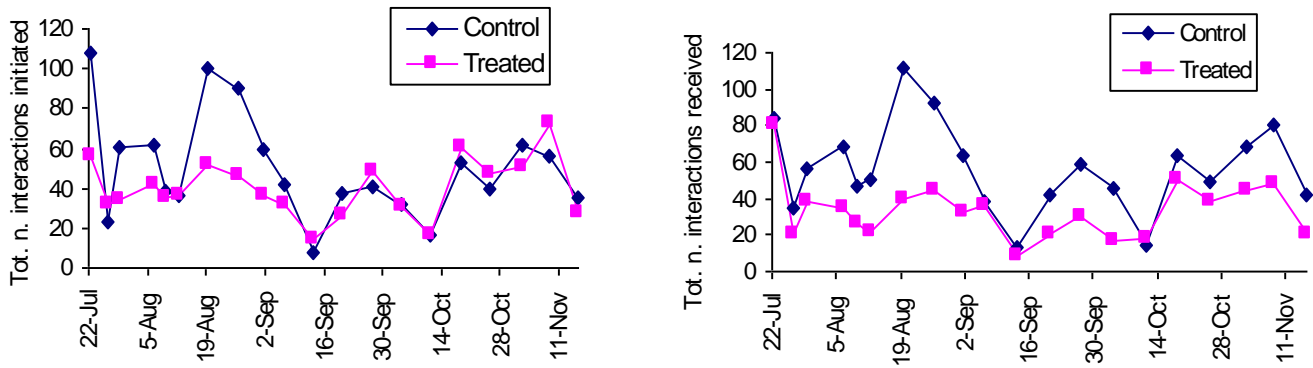


Figure 3. Total number of agonistic interactions initiated and received by control and treated wild boar females. Treatment with the GnRH vaccine occurred on 16th and 17th August.

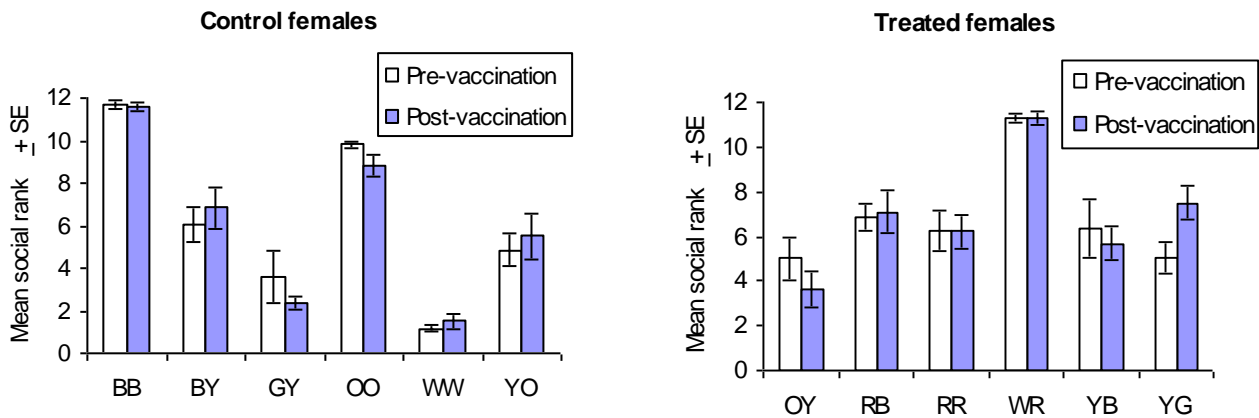


Figure 4. Social rank of individual wild boar females (control and treated group) before and after treatment with the GnRH vaccine. Codes reported on the x axis refer to the identity code of each sow (e.g. BB=blue-blue; OY =orange-yellow, etc.).

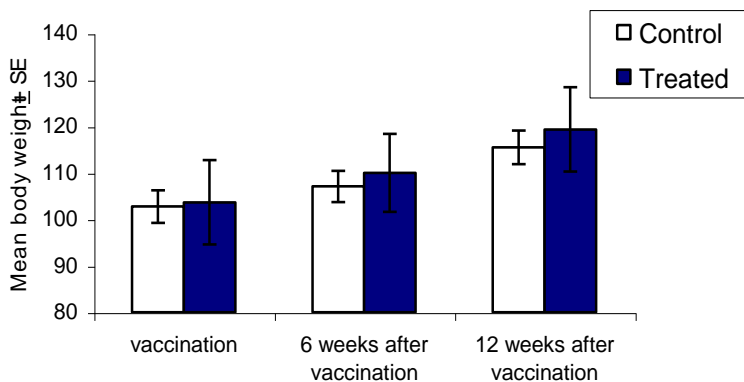


Figure 5. Body weight of wild boar females at vaccination and 6 and 12 weeks after vaccination.

The PCA on the biochemical variables showed that the first PC explained 32.2 % and the second PC 17.5 % of the variability. The factors that contributed most to the first PC were alpha-globulins, ionised calcium, albumin, creatinine, and inorganic phosphate. The REML indicated that biochemical values were affected by time ($\text{Chi-sq.}=193.05$, $d.f. = 2$, $P < 0.001$) but not by treatment ($\text{Chi-sq.}=1.57$, $d.f. = 1$, $P > 0.05$) or by time*treatment interaction ($\text{Chi-sq.}=0.13$, $d.f. = 2$, $P > 0.05$) (Figure 6).

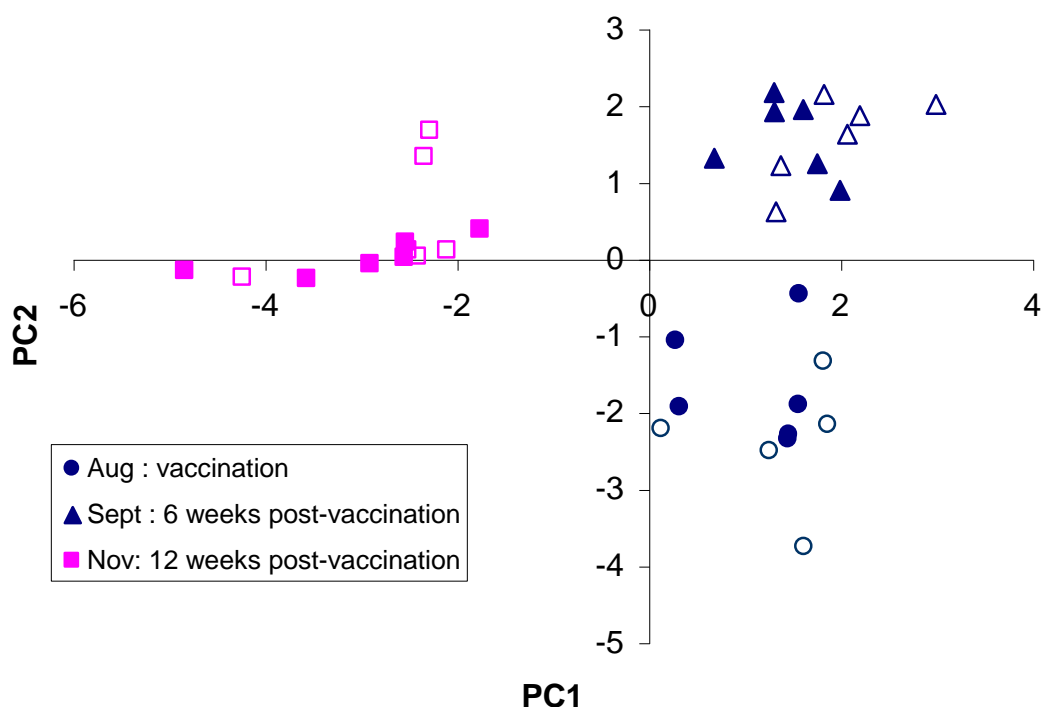


Figure 6. Principal Component Analysis on 15 biochemical variables derived from blood samples of treated (open symbols) and control (full symbols) wild boar females at vaccination and 6 and 12 weeks after vaccination.

The PCA on the haematological variables showed that the first PC explained 39.7 % and the second PC 17.2 % of the variability. The factors that contributed most to the first PC were haemoglobin, PCV%, RBC, WBC and Neutrophils. The REML indicated that the haematological values were not affected by time ($\text{Chi-sq.}=1.22$, $d.f. = 2$, $P > 0.050$, treatment ($\text{Chi-sq.}=0.02$, $d.f. = 1$, $P > 0.05$) or time*treatment interaction ($\text{Chi-sq.}=0.19$, $d.f. = 2$, $P > 0.05$) (Figure 7).

Of all the biochemical and haematological variables that were tested for differences between treated and control females at 6 and 12 weeks after vaccination only the Beta-globulins differed 6 weeks after vaccination (mean = 11.7 ± 3.2 SE for control and 19.4 ± 0.99 for treated females), indicating an immune response in treated females.

In both treated and control groups the concentration of cortisol was higher before than after vaccination (Figure 8). This was partly due to pregnancy in six of the boar and partly to other factors such as handling of the animals, anaesthesia and disturbance due to the removal of the piglets (see discussion). Due to these confounding factors the cortisol levels in the pre-vaccination period may not have been representative of basal conditions and thus were not used as a comparative welfare measure for the post-vaccination period. Hence the assessment of the impact of the vaccination on the cortisol levels in these animals was addressed by comparing the relative levels of this hormone in the treated and control groups post-vaccination but pre-mating (i.e. from weeks 1 to 13). In the post-vaccination period (weeks 1 to 13) cortisol levels changed with date ($\text{Chi-sq.} = 3.47$, $d.f. = 31$, $P < 0.001$) but not with treatment ($\text{Chi-sq.} = 0.05$, $d.f. = 1$, $P > 0.05$) or date* treatment (Chi-sq.

= 0.66, *d.f.* = 28, *P* > 0.05). These results suggested that the vaccine had no effect on the cortisol in the 13 weeks following vaccination.

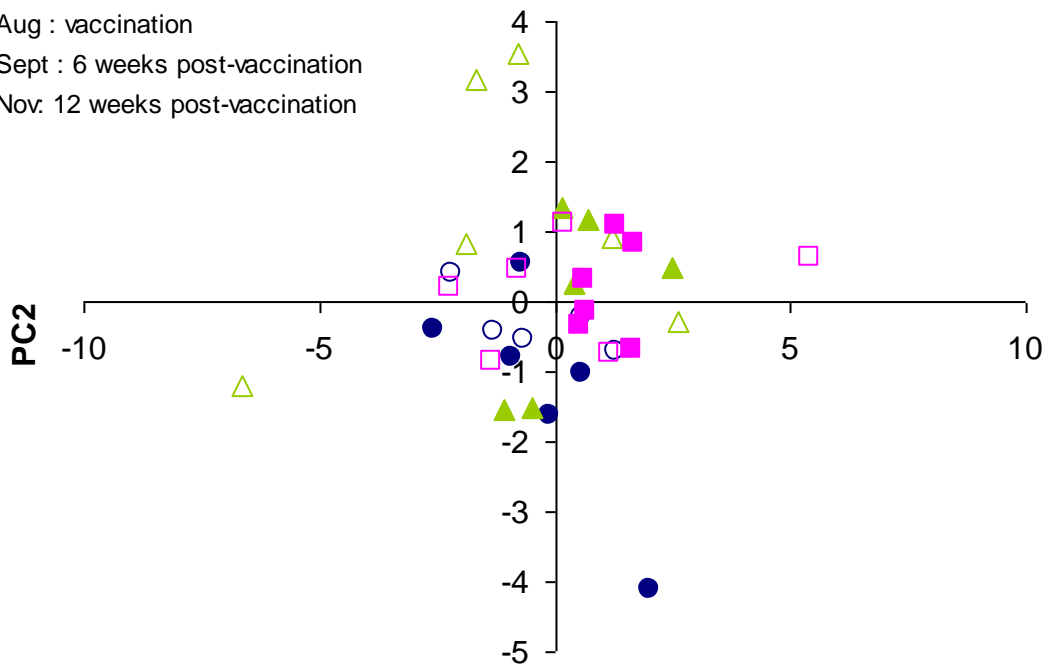


Figure 7. Principal Component Analysis on 10 haematological variables derived from blood samples of treated (open symbols) and control (full symbols) wild boar females at vaccination and 6 and 12 weeks after vaccination.

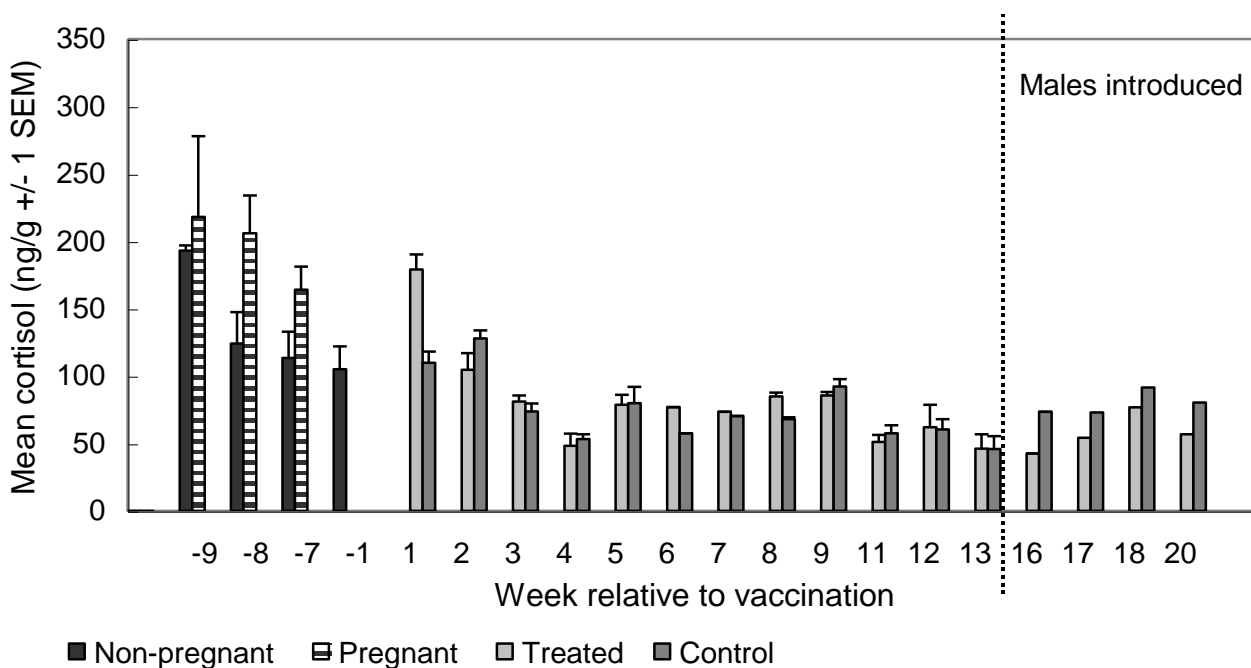


Figure 8. Mean faecal cortisol (ng/g dry faeces) for pregnant (N=6) and non-pregnant wild boar (N=6) before vaccination and control (N=6) and treated boar (N=6) after vaccination.

4. Discussion

This study showed that the GnRH vaccine can significantly reduce the reproductive output of individual wild boar with no appreciable side-effects in the three months following vaccination. When tested in deer, coyotes, feral pigs and bison the single-injection GnRH vaccine caused infertility for a minimum of one or two years (Miller *et al.* 2000, Miller & Killian 2000, Curtis *et al.* 2002). GnRH contraceptive vaccines have also been evaluated as immunocastration agents in pets and farm animals (e.g. Adams & Adams 1986, Ladd *et al.* 1994, Levy *et al.* 2004). However, the present study is the first comprehensive investigation of the potential side effects of this vaccine on the physiology and behaviour of treated animals, as well as the vaccine's effectiveness.

Faecal progesterone levels in the treated boar did not fall until 12 weeks post-vaccination suggesting that this is the time lag before the effects of the vaccine become fully developed. Thereafter, the levels of progesterone were maintained at low levels in treated sows but did not drop to zero at any point during the course of this study. The fact that the levels of progesterone were consistently low but nonetheless detectable indicates that treatment with the GnRH vaccine suppressed but did not completely block progesterone production. These measurable progesterone levels suggest that other steroid hormones (e.g. oestrogen) would be similarly affected. The fact that such hormones may still be present after treatment with the GnRH vaccine has positive implications for the welfare of these animals, since complete absence of these important hormones might have more wide ranging effects on animal health and welfare. The reduction of faecal progesterone in treated sows occurred approximately five weeks after the antibody titres had reached maximal levels, which may be the period required for clearance of pre-circulating levels of this hormone.

The faecal progesterone ELISA method developed for this study was able to detect significantly elevated progesterone in the faeces of pregnant wild boar, suggesting that this is a viable method of pregnancy detection in this species. The detection of pregnancy was 100% accurate, with positive results being gained as early as 14 days after mating was observed. This method is particularly effective since no other documented non-invasive technique in porcine species can detect pregnancy at such an early stage (e.g. Moriyoshi *et al.* 1997, Isobe & Nakao 2004). The development of this technique has both practical and welfare benefits since, unlike other methods, it requires no anaesthesia, no veterinary input and is simple and inexpensive.

Due to practical difficulties and safety concerns associated with faecal sample collection during certain periods (e.g. when boar were littering) insufficient samples were collected to determine individual oestrous cycles. However, low and high levels of progesterone, perhaps corresponding to the different phases of the oestrus cycle, were found in the boar suggesting that the animals were ovulating before treatment. Consistently low progesterone in non-pregnant sows between June and mid-September may indicate anoestrus since boar in the wild are known to have an anoestrous period in the summer (Maugert 1982). Similarly, the increase in progesterone in late September (i.e. around 5 weeks after vaccination) is consistent with the onset of the cycling in free-living wild boar (Maugert *et al.* 1984).

Throughout the course of this study, no differences in time budgets were observed during the pre- and post-vaccination periods between treated and control females, indicating that, at least up to 13 weeks following treatment, the GnRH vaccine did not affect the behaviour of treated sows. The time spent in different activities by both treated and control females changed significantly with date as all animals spent progressively more time in activities such as feeding and walking and less time sleeping. These results might have been biased by observation sessions being limited to only three morning hours per day. However, a few pilot sessions carried out in the afternoons indicated that time budgets at this time did not differ from those measured during morning sessions. In future studies, fitting individuals with collars equipped with activity switches will allow 24 hour monitoring of activity, although not of full behavioural time budgets. A strong indication that the data collected here reflected natural changes in the time budgets of wild boar is that they closely matched findings from previous studies carried out on free-living animals. Here time budgets have been observed to follow seasonal patterns related to factors such as temperature, humidity, day length, food availability and mating. For instance,

Mauget *et al.* (1984) showed that in summer wild boar spent about 25 % of their time feeding compared to 33% in autumn, whilst the time spent sleeping and resting decreased from 58% in summer to 52% in autumn. Similarly, Janeau & Spitz (1984) and Gerard & Campan (1988) indicated that in autumn wild boar spend relatively more time eating and less time resting compared with summer months.

In the present study, the total number of agonistic interactions initiated and received by treated and control females decreased with time, although the social ranks remained stable throughout the study. These results agree with previous studies indicating that after an initial period, during which the boar in a group establish dominance ranks, the hierarchy becomes stable (Graves 1984). These results also suggest that, at least in the short-term (13 weeks after vaccination), no appreciable effects of the GnRH vaccine on the behaviour of treated sows emerged.

No differences in blood parameters were observed between treated and control sows indicating that the GnRH vaccine did not affect the health profiles of wild boar. The date of collection affected the biochemistry but not the haematology of both groups and showed that some biochemical variables might vary seasonally.

The only indication of an effect of the GnRH vaccine on the physiology of wild boar was an increase in body weight that was more pronounced in treated than in control females. Similar body weight increases have been reported for GnRH-treated male pigs compared to control pigs (Cronin *et al.* 2003). This trend, if continued, could potentially lead to long-term health and welfare problems associated with obesity in treated animals, although such an effect might be less likely with free-living animals rather than the captive individuals reported on here that had access to abundant food.

Consistent with the results obtained on behaviour, the data on cortisol confirmed that the levels of this hormone did not differ in treated and control groups during the post-vaccination period. Cortisol is known as the 'stress hormone' because, unlike other hormones also associated with stress (e.g. adrenaline and endorphins), the concentration of cortisol does not increase during non-stress-related events such as mild or moderate exercise (Few *et al.* 1974). Combined with behavioural observations, cortisol can be used as a measure of welfare. Faecal cortisol can be a particularly useful indicator of chronic, long-term stress. Measuring cortisol in faeces has the added advantage of allowing non-invasive sampling, negating the need to anaesthetise, handle and disturb the study animals, which could affect the parameters being measured. In this study, practical and safety issues constrained collection of faeces and samples were not collected when litters were present and for a few weeks after the male boar were introduced. This has resulted in gaps in the data, in particular in weeks -7 to -1 when the boar were littering. However, the similar levels of cortisol found in treated and control groups suggested that the GnRH caused no additional stress to the treated group for the three months following vaccination. It is important that long-term studies are carried out on these animals to establish whether the absence of any effect of the GnRH vaccine on cortisol levels persists. In this study the control group were administered the adjuvant (without the vaccine), and thus acted as controls for the effects of the vaccine rather than the adjuvant, although behavioural observations showed no obvious signs of distress or pain (e.g. limping, hunched posture). To assess potential welfare effects of the adjuvant alone future studies might also include a control group administered with saline only.

Relatively high levels of faecal cortisol were found in all boar at the beginning of the study. This is, in part, likely to be due to the stress of translocation, new surroundings, addition of unfamiliar conspecifics to the group structure and changes in daily routine. In particular, research techniques (e.g. anaesthesia and blood sampling) may have had a profound effect on the animals and might have increased the length of time required for the animals to habituate to their new surroundings. In addition, six of the boar were pregnant on arrival at CSL and this would have significant effects on the cortisol levels of these animals. Elevated cortisol is known to be associated with pregnancy and in particular with late gestation (e.g. Weingrill *et al.* 2004, Smith & French 1997, Ziegler *et al.* 1995, Lockwood *et al.* 1996). This gestation-linked increase in cortisol is thought to be due to two factors; firstly the Hypothalamus Pituitary Adrenal (HPA) axis may be stimulated during pregnancy by higher levels of oestrogens causing an increase in the concentration of other steroid hormones (Coe *et al.* 1986); secondly, the placenta itself releases Corticotrophin Releasing Hormone, the precursor of cortisol. The fact that six of the boar were in late gestation could also have had an impact on the cortisol levels of the non-pregnant boar. Pregnancy and littering of their conspecifics might have effects on the behaviour and

interactions of the boar as a group. In this study, pregnancy also significantly affected the management and husbandry of the group (e.g. increased presence of staff, veterinary surgeon and anaesthesia of mothers) causing unpredictability in their daily routine, which is known to increase stress levels.

This study found that the GnRH vaccine renders individual wild boar sows infertile, at least in the short-term, with no other measurable effects on the behaviour, welfare and physiology of treated animals except an increase in body weight. Increasing numbers of theoretical and empirical models (McLeod & Saunders 200; Smith & Cheeseman 2002; Shi *et al.* 2002) indicate that fertility control could be as effective as lethal control for reducing overabundant populations. If future research confirmed the initial findings reported here then the use of the GnRH vaccine might become a realistic option to manage free-living populations of wild boar as well as other mammalian species. Currently this would be limited to applications where delivery by injection is feasible. However, the development of effective oral vaccines, along with species-specific methods of delivering these, would expand the range of potential practical applications. Wildlife management is a particularly sensitive area in which opinions tend to be polarised. Against this challenging background effective fertility control methods would provide Defra with (i) the possibility of advocating non-lethal methods for managing overabundant populations and (ii) enhanced objective decision making about best practice for wildlife management. However, in order to build on this project's very encouraging proof of concept in this model species, using emerging technology in captive animals, studies of free-living animals are required to confirm the long-term effectiveness and humaneness of the technique. Confirmation in such studies, of the promise reported here, would offer real prospects of practical applications being realised for fertility control, with the potential to revolutionise our approach to wildlife management in the UK. A programme of research has thus been recommended to Defra that builds on the success of this project. The objectives of this proposed research are:

1. Investigate the long-term effectiveness and possible side effects of the GnRH vaccine on captive wild boar.
2. Assess the effectiveness and potential side effects of GnRH vaccine on individual free-living wild boar.
3. Evaluate the effectiveness and potential behavioural and welfare effects of one avian contraceptive in the Rose-ringed parakeet as a model species.
4. Develop cost-effective systems to deliver fertility control agents to target species.
5. Establish requirements for evaluation of population level effects of fertility control agents.

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