PE1.08 Effects of vaccination with Improvac® on boar taint and carcase quality of male pigs reared under commercial conditions in Europe 89.00

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***Abstract*—to determine the efficacy of a vaccine with a gonadotrophin releasing factor (GnRF) analogue conjugated to a carrier protein (Improvac®, Pfizer Animal Health) for the reduction of boar taint in male pigs, studies were conducted at commercial premises in five European countries with a prospective parallel design. 1450 neonatal pigs were randomised to one of three treatments: 535 pigs for physical castration (T1), 639 pigs for vaccination with Improvac (T2) and 276 pigs to be left entire (T3). Pigs in group T2 received two doses of vaccine at an interval of 4-6 weeks with the second at least 4 weeks before slaughter. Serum samples were analysed for antibodies against GnRF and testosterone, testicles were measured, and body weights were recorded at key time points. At slaughter, the presence of the boar taint compounds androstenone, skatole and indole in belly fat was determined. Carcase weight and fat content were assessed at slaughter. Compared with barrows, vaccinated pigs had similar average daily weight gain and similar slaughter weights. At slaughter, boar taint compounds were well controlled in barrows and vaccinates but were below accepted thresholds for human detection in only 43% of entire pigs. Carcases of vaccinates contained less backfat than barrows (*P*<0.005). Improvac was highly effective in the reduction of detectable boar taint with a trend towards improved carcase quality.**

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I. INTRODUCTION

lthough there is considerable individual variation, Athe compounds responsible for boar taint in pig meat are detectable by a high proportion (50–95%) of the human population [1,2]. Taint is a major cause of consumer dissatisfaction [3] and although it can be alleviated to a degree by changes in management such as the slaughter of pigs at an earlier age, the traditional strategy has been physical castration. However, welfare concerns have led the swine industry to explore alternative solutions with the intention of producing the highest quality meat for human consumption whilst optimising animal welfare. One novel and promising solution is the immunisation of male pigs against endogenous gonadotrophin releasing factor (GnRF) which results in suppression of normal sexual function [4]. The objective of the present studies was to determine the field efficacy of immunisation against GnRF using a commercial vaccine (Improvac®, Pfizer Animal Health) for the reduction of boar taint and associated effects on carcase quality when used within current European production systems.

II. MATERIALS AND METHODS

These studies were conducted at seven sites in five countries across Europe in 2005 and 2006: Spain (459 pigs), Germany (372 pigs), Denmark (172 pigs), Hungary (249 pigs) and the United Kingdom (198 pigs). The protocols were subject to prior ethical review and animal welfare was maintained throughout, in accordance with regulatory requirements. All study participants were required to give their informed consent.

PROCEDURE

a. Design

Pigs were allocated to treatment using a computer generated randomisation plan for each site, blocked by order of enrolment, within a prospective, randomised complete block, parallel group design. Male piglets were allocated to one of three treatments, physical castration (T1), vaccinated with Improvac (T2), and negative control (T3). In the UK, no pigs were physically castrated and pigs were allocated to T2 and T3 only. Timing of vaccination was based on the predicted day of slaughter. Pigs were slaughtered at age 21–24 weeks at commercial abattoirs and processed for human consumption. All personnel responsible for carcase quality assessments and laboratory sample analyses were blinded to treatment.

b. Treatments

Neonatal pigs allocated to group T1 were physically castrated by experienced personnel on the day of enrolment. Pigs allocated to group T2 received two doses of Improvac (V1 and V2) at an interval of 4–6 weeks, with V2 at least 4 weeks prior to slaughter (S). Pigs allocated to T3 were left entire and untreated except in the UK, where they received placebo injections of physiologically normal saline on vaccination days. Vaccine and saline were administered by subcutaneous injection behind the left (V1) and right (V2) ears. Each 2 mL dose of vaccine contained 150 µg GnRF conjugate per mL, except in the UK where vaccine with an antigen content of 101 µg/mL was used.

c. Animal husbandry

Pigs were identified by uniquely numbered ear tags. Pigs enrolled in the studies were farrowed on site, and standard site specific management procedures for piglets—which varied according to local veterinary advice—were followed, including iron supplementation, teeth clipping, and vaccination against *Mycoplasma hyopneumoniae*. Prior to enrolment, piglets were clinically examined to confirm the presence of two normal testicles and suitability for inclusion. Subsequently, pigs were examined daily for routine health. In Spain and Germany, sub-sets of pigs in T1 and T2 were given detailed clinical examinations for 5 days after castration and vaccination. At any point during the study, pigs were withdrawn if they met pre-defined criteria of being underweight, in poor or declining health, failing to respond to medication, receiving immunosuppressive treatment within four weeks of vaccination, or the presence of testicles in T1 pigs or absence of testicles in pigs in T2 or T3.

Commercial hybrid pigs were used at each site which included Landrace, Yorkshire, Large White, Hampshire, Duroc, Piétrain, and Bundeshybridzuchtprogramm hybrids. Accommodation varied between sites depending on pre-existing housing arrangements. Feed was appropriate for age but differed between sites. At each site, pigs in each group were fed the same ration. Food and water were available *ad libitum*.

d. Samples and measurements

During clinical examinations rectal temperatures were recorded and injection sites were measured and scored 1 (negligible) to 4 (large). Pigs were weighed at all sites—except in the UK—at enrolment, weaning, V1, V2, V2+14 days and S–1 day. For pigs in T2 and T3 in Germany, Denmark and Hungary (T2 only in Spain) testicle dimensions were measured at S or S–1 day.

Blood samples were collected at V1, V2, V2+14 days, and at S–1 day. Following collection, blood was allowed to clot prior to being centrifuged and the sera harvested.

Following slaughter, the hot carcase weight (or prepared carcase weight in Denmark) was recorded at the abattoir. The depth of backfat at the P2 level was measured and a sample of at least 10 g of belly fat was collected from around the second and third nipple, from each pig. Samples of sera and belly fat were stored below –15°C prior to batch processing. In Spain, Germany and Hungary, carcases were graded using the SEUROP grading system. In Denmark, percentage meat content of specific joints was evaluated. Carcases were not assessed in the UK.

e. Sample analyses

All analyses were conducted in accordance with GLP guidelines [5]. Each analytical methodology was validated and blank porcine serum and belly fat were used as reference samples.

Serum samples were analysed for IgG antibodies against GnRF using an indirect, non-competitive ELISA

Assays of serum testosterone were performed using HPLC with mass spectrometric detection. The lower limit of quantification (LLOQ) was 0.1 ng/mL of serum.

Belly fat androstenone was also determined using HPLC with mass spectrometric detection. Skatole and indole assays were performed using HPLC with fluorescence detection. The LLOQs for androstenone, skatole and indole were 200 ng/g, 13.2-20 ng/g and 7.05-12.9 ng/g, respectively.

f. Statistical methods

The pig was the experimental unit until weaning and segregation of the groups, after which the fattening pen was the experimental unit. Statistical comparisons were made between T1 and T2, except in the UK where the comparison was between T2 and T3. Average daily weight gains were calculated for each time period and analysed using linear mixed models with treatment as a fixed effect, and fattening pen and block within fattening pen as random effects. GnRF and serum testosterone levels were summarised and Fisher's exact tests were used to compare the frequency of results

below the LLOQ; quantifiable data were summarised using geometric means for each treatment group. Data from the analyses of taint compounds, carcase weights, and backfat thicknesses were summarised and least squares means were compared between treatments using a linear mixed model with treatment as a fixed effect and fattening pen and block within fattening pen as random effects. Androstenone and skatole results were stratified by concentration at established cut-off levels and cross-tabulated.

III. RESULTS AND DISCUSSION

Of the 1450 pigs enrolled, 9.3% (50/535) in T1, 4.7% (30/639) in T2, and 5.8% (16/276) in T3) died or were euthanased during the study. A further 49 pigs (23 in T1, 18 in T2, eight in T3) were withdrawn. Reasons for mortality or withdrawal included a range of common conditions affecting growing pigs. Amongst the withdrawals were 19 pigs at one site in Spain, withdrawn prior to slaughter for being below the minimum acceptable body weight for the abattoir (13 pigs in T1 and six in T2). Other clinical abnormalities occurred in similar numbers across the groups and appeared to be unrelated to treatment. Post-castration, nine pigs developed local infections or septicaemia. In Spain, 38% of monitored pigs in T1 had a rectal temperature >40ºC for 1–2 days post-castration compared with 32% of monitored pigs in T2.

During the study, clinically significant reactions at the injection site were observed in four pigs in Spain, two pigs in the UK, and up to 28% of pigs in Germany and Denmark. Mild reactions were observed in 30% of pigs in Spain. The association with particular study sites suggests variation in either the vaccination or assessment techniques. Most reactions were mild and resolved rapidly, confirming that the aqueous formulation is generally well tolerated.

There were no significant differences between T1 and T2 in the level of quantifiable antibodies against GnRF at V1, however, there were significant differences between T1 and T2 at V2, V2+14 days and at slaughter ($P<0.0001$). For vaccinated pigs, mean levels of GnRF antibody compared with V1 were up to 2-fold higher at V2, 16 to 30-fold higher at V2+14 days but at slaughter had halved to 8 to 15-fold higher than at V1. For vaccinates, mean levels of testosterone showed little change from V1 to V2 but had declined by half at slaughter. In contrast, mean testosterone levels for entire pigs (in Hungary and UK) had increased 1.5 to 3-fold from the time of V1 to slaughter. The data indicate that for clinical effect the second injection is required to give an anamnestic antibody response, with maximal effect being observed at V2+14. However, the effect is of limited duration requiring the second injection to be no more than a few weeks before slaughter. At slaughter, testicle dimensions were generally smaller by 20–40% among pigs in group T2 compared with T3, indicating that testicle size may have a utility in screening for successful vaccination. However, because of the variation between pigs, testicle size may not be suitable as an indicator of successful vaccination in individual pigs.

At slaughter, belly fat androstenone concentrations were below LLOQ in all pigs except one in T1 and 97% in T2, but only 18% of boars in T3. Levels of skatole in belly fat were below LLOQ for 21% of pigs in T1 and T2, and 17% in T3. Results for indole were variable between sites, with inconsistent differences between groups. Among pigs with quantifiable levels of androstenone and skatole, stratification of carcases into concentration ranges known to represent acceptable thresholds for taint showed a demonstrable effect of vaccination on the reduction of the incidence of taint compared with entire pigs (Table 1). None of the carcases in T1 and only six (1%) in T2 were categorised as potentially tainted compared with 57% in T3, using cut-off levels of 200 ng/g skatole and 500 and 1000 ng/g androstenone [3]. None of the vaccinated pigs were in the high risk category for either androstenone or skatole.

These studies necessarily involved some departures from normal commercial practices. The protocols and monitoring ensured that both vaccination and castration were well conducted. Cryptorchid and intersex pigs—occasional causes of boar taint in countries where physical castration is normally used—were excluded from these studies. Also, following vaccination there was no assessment of vaccinated pigs to identify those needing redosing because of persistent entire male behaviour, which is recommended procedure.

There were differences in age at slaughter between countries, but within each country the mean body weights at slaughter were comparable

between treatment groups (Table 2). At V1, V2, and V2+14 days the mean body weights of pigs in T1, T2 and T3 were also comparable. Similarly, least squares mean daily weight gains from enrolment to slaughter showed no significant differences between treatments (Table 2). In Germany, least squares mean daily weight gains were significantly higher for T2 compared with T1, from V1 and from V2 to slaughter (P<0.05).

There were no treatment related differences between least squares mean hot (or prepared, Denmark) carcase weights (Table 2), although there was a significant site effect on least squares mean hot carcase weight in Germany (P<0.0001). Analysis of least squares mean backfat thickness at the level of P2, showed that pigs in T2 had significantly less backfat than pigs in T1, in Spain (P<0.0001) and Germany (P=0.0021). Although, where analysed treatment differences were not statistically significant, carcase grading showed a trend for vaccinates to have a higher percentage of lean meat compared with barrows, but lower than for entire pigs (Tables 3 and 4).

These results replicate earlier data from Australia [4], and demonstrate the efficacy of Improvac, even at the lower titre utilised at the UK site. European legislation is trending towards restrictions on the routine use of physical castration of piglets, and there is increased interest in production systems with lower slaughter weights to minimise risk of taint. At the same time, consumers show increasing preference for lean meat and appreciation of premium meat products with an emphasis on flavour, achieved by rearing pigs to an older slaughter age to improve the cooked meat flavour. Vaccination offers the potential to satisfactorily reduce meat taint and improve welfare, without having to reduce the slaughter age and compromise production of premium meat products.

IV. Conclusion

Vaccination against endogenous GnRF resulted in a marked reduction in the boar taint compounds androsterone and skatole. Carcase quality was generally intermediate between barrows and entire boars with a trend for less fat and more lean meat compared with barrows.

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Table 1. Boar taint: percentage of animals in each treatment within each level of taint.

Skatole Concentration (ng/g)	Androstenone Concentration (ng/g)								
	<500			500–1000			>1000		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
<100	97 % *n*=440	95 % *n*=549	36 % *n*=90	–	0.5 % *n*=3	20 % *n*=50	–	–	16 % *n*=39
100-200	3 % *n*=13	4 % *n*=23	7 % *n*=17	–	-	6 % *n*=14	–	–	5 % *n*=13
>200	–	0.3 % *n*=2	3 % *n*=8	–	0.2 % *n*=1	2 % *n*=5	–	–	6 % *n*=15

Acceptable taint compound cut-offs set at 200 ng/g skatole and 500–1000 ng/g androstenone [3].
T1 = castrates, T2 = vaccinates, T3 = entire males. *n*=number of pigs.

Table 2. Performance evaluations.

	Spain			Germany			Denmark			Hungary		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Mean body weight ± SD (kg)*	94.7 ±14.2 *n*=198	94.3 ±14.2 *n*=197	NA	128 ±14.8 *n*=143	128 ±13.0 *n*=154	127 ±9.3 *n*=17	118 ±13.5 *n*=63	120 ±12.8 *n*=61	117 ±11.1 *n*=28	127 ±15.7 *n*=63	127 ±22.4 *n*=75	130 ±18.9 *n*=71
LS mean daily weight gain (SE) (g/day)†	568 (12.2) *n*=198	564 (12.5) *n*=197	NA	657 (10.4) *n*=143	655 (10.2) *n*=154	NA	791 (24.2) *n*=63	805 (24.2) *n*=61	786§ ±67.0 *n*=28	605 (11.4) *n*=63	604 (10.4) *n*=75	620 (10.7) *n*=71
LS mean hot carcase weight (SE) (kg)‡	72.2 (1.28) *n*=178	69.2 (1.29) *n*=191	74.2§ ±10.6 *n*=37	102 (1.37) *n*=151	99.8 (1.36) *n*=158	96.4§ ±7.2 *n*=17	90.2 (3.35) *n*=62	89.3 (3.34) *n*=61	88.5§ ±9.1 *n*=28	101§ ±13.3 *n*=62	97.8§ ±16.4 *n*=73	102§ ±15.3 *n*=71
LS mean P2 backfat thickness (SE) (mm)	20.1 (0.59) *n*=178	15.9 (0.59) *n*=190	14.8§ ±5.9 *n*=37	20.9 (0.69) *n*=105	16.2 (0.67) *n*=110	14.7§ ±1.8 *n*=11	NA	NA	NA	21.7§ ±6.2 *n*=63	17.8§ ±4.4 *n*=75	17.6§ ±4.4 *n*=71

*On the day before slaughter. †From enrolment to slaughter. §Arithmetic mean ± SD. ‡In Denmark measured as the prepared carcase weight. T1 = castrates, T2 = vaccinates, T3 = entire males. SD=standard deviation. SE=standard error. *n*=number of pigs. NA = not available. P2 is defined as 6 cm off the carcass midline between the third and fourth last ribs.

Table 3. SEUROP carcase grade in Germany, Hungary and Spain*:
Percentage of pigs in each grade.

Carcase Grade	T1	T2	T3
S (>60% lean)	–	–	1 % *n*=1
E (55 to <60% lean)	22 % *n*=81	57 % *n*=216	68 % *n*=80
U (50 to <55% lean)	36 % *n*=132	20 % *n*=76	14 % *n*=17
R (45 to <50% lean)	22 % *n*=79	11 % *n*=42	8 % *n*=10
O (40 to <45% lean)	12 % *n*=43	7 % *n*=25	5 % *n*=6
P (<40% lean)	8 % *n*=28	5 % *n*=20	3 % *n*=4

* In Spain, 81 pigs < 60 kg or >90 kg body weight (29in T1, 45 in T2, 7 in T3) excluded from table.
T1 = castrates, T2 = vaccinates, T3 = entire males. *n*=number of pigs.

Table 4. Carcase evaluation in Denmark:
least squares mean meat percentage (SE)

	Meat	Ham meat	Middle meat	Front meat
T1	58.4 (0.35) *n*=61	71.1 (0.36) *n*=51	62.2 (0.46) *n*=59	67.0 (0.36) *n*=51
T2	59.1 (0.35) *n*=61	71.7 (0.36) *n*=48	63.3 (0.45) *n*=60	67.3 (0.37) *n*=48
T3§	60.8 ± 2.3 *n*=28	73.8 ± 2.0 *n*=27	65.7 ± 2.8 *n*=28	68.9 ± 2.0 *n*=27

T1 = castrates, T2 = vaccinates, T3 = entire males. SE=standard error.
n=number of pigs. §Arithmetic mean ± SD.