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Safety and efficacy of a new octavalent combined Erysipelas, Parvo and Leptospira vaccine in gilts against *Leptospira interrogans* serovar Pomona associated disease and foetal death

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ABSTRACT

The safety and protective efficacy of a new octavalent combination vaccine containing inactivated *Erysipelothrix rhusiopathiae*, Parvovirus, and *Leptospira interrogans (sensu lato)* serogroups Canicola, Icterohaemorrhagiae, Australis (Bratislava), Grippotyphosa, Pomona and Tarassovi – Porcilis® Ery + Parvo + Lepto – was evaluated in laboratory studies and under field conditions.

The safety (2× overdose and repeated dose) was tested in 26 gilts. In this study, neither vaccine related temperature increase nor other systemic reactions were observed after intramuscular vaccination. No local reactions were observed except for one animal that had a small local reaction (2 cm diameter) that lasted for 5 days after the third vaccination.

Efficacy was tested in 40 gilts. A group of 20 gilts was vaccinated at 20 and 24 weeks of age with Porcilis® Ery + Parvo + Lepto and a group of 20 age- and source-matched animals served as the control group. The gilts were inseminated at 41 weeks or 66 weeks of age and were challenged with serovar Pomona 10 weeks after insemination, corresponding to 6 months ($n = 2 \times 10$) and 12 months ($n = 2 \times 10$) after the last vaccination. After both the 6- and 12-month challenges the control animals developed clinical signs (fever, lethargy and anorexia) and leptospiraemia as determined by positive blood culture. In addition, both the 6- and 12-month challenges resulted in death of 21% and 27% of the total number of foetuses in the control groups, respectively. Clinical signs and leptospiraemia were statistically significantly lower in vaccinated gilts after both the 6- and 12-month challenges. In addition, foetal death was statistically significantly lower (3% and 2%, respectively) in vaccinated gilts after both the 6- and 12 month challenges.

The vaccine was tested further under field conditions on a Portuguese farm with a history of an increasing abortion rate associated with a *Leptospira* serovar Pomona infection (confirmed by PCR and serology). This study was designed as an observational-longitudinal field study. At the start of the study, all breeding sows and replacement gilts on the farm were vaccinated twice with Porcilis® Ery + Parvo + Lepto at an interval of 4 weeks. Starting six months after the primary vaccination schedule, the animals were re-vaccinated during the second week of every subsequent lactation. New replacement gilts were vaccinated using the same schedule. After vaccination, the abortion rate reduced rapidly from 12.6% in winter months of 2012 (December 2011 to March 2012) to 0.5% in winter months of 2013, a statistical significant decrease of 96%. The total number of abortions on the farm decreased from 55 in 2012 to 6 in 2013. Thereafter, the abortion rate remained stable and in the period December 2013 to April 2014 was still low (0.6%).

In conclusion, the present studies demonstrate that the octavalent Porcilis® Ery + Parvo + Lepto vaccine can be safely used in gilts and sows and induces significant protection, for the duration of at least one year, against serovar Pomona induced clinical signs, leptospiraemia and foetal death. Protection against Pomona associated reproductive failure was confirmed under field conditions where a significant reduction in abortion rate was observed.

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1. Introduction

Swine erysipelas is an economically important disease worldwide. It is caused by the bacterium *Erysipelothrix rhusiopathiae* and is manifested as acute or subacute septicaemia and chronic proliferative lesions. The acute form is characterized by typical rhomboid (diamond-shaped), urticarial skin lesions and fever, whereas the chronic form is characterized by arthritis [1].

Porcine parvovirus is a common and important cause of infectious infertility and has been found to be present in the majority of pig herds. Parvovirus infection in adult pigs rarely, if ever, causes clinical signs. However, the virus readily crosses the placental barrier with lethal effects on the embryos and foetuses in susceptible pregnant animals. Depending on the stage of pregnancy, this will result in resorption and return to heat or mummification of the embryos, which will be delivered at term together with a variable number of weak or healthy piglets [2].

Leptospirosis, caused by *Leptospira interrogans sensu lato*, is also a cause of reproductive failure in pigs worldwide, which manifests itself as abortions or the birth at term of a variable number of mummified, autolysed, stillborn and/or weak piglets [3–5]. Leptospirosis in pigs, as in other animals and humans, is difficult to diagnose and its incidence is most probably underestimated. Culture, serology and PCR often are negative even when active infection is present. In addition, in sows, the clinical signs of *Leptospira* infection are few, vague and non-specific or absent [3–5].

Vaccines against Erysipelas and Parvovirus are routinely used in the pig industry whereas leptospirosis vaccines are used less commonly. For convenience of the user and to reduce the number of injections given to gilts and sows, a ready-to-use combination product including *Leptospira* would be highly desirable. Therefore, we developed a new, ready-to-use combination product by adding six relevant swine *Leptospira* antigens (serogroups Canicola, Icterohaemorrhagiae, Australis (Bratislava), Grippotyphosa, Pomona and Tarassovi) to an existing vaccine, Porcilis® Ery + Parvo. This new combination vaccine was subsequently tested for safety and efficacy against each of the infectious agents. The present study focuses on the demonstration of vaccine efficacy against Pomona, the most notorious *Leptospira* serovar associated with foetal death [6], under controlled laboratory and field conditions.

2. Materials and methods

2.1. Vaccines

A vaccine containing inactivated *E. rhusiopathiae*, Parvovirus, *L. interrogans (sensu lato)* serogroups Canicola, Icterohaemorrhagiae, Australis (Bratislava), Grippotyphosa, Pomona and Tarassovi and the Diluvac Forte® adjuvant (Porcilis® Ery + Parvo + Lepto, MSD Animal Health). For vaccine preparation a mixture of the eight antigens at proper concentration was 1:1 mixed with double concentrated Diluvac Forte® adjuvant. The Diluvac Forte Adjuvant is a Vitamin E based oil (non-mineral oil) in water emulsion.

A vaccine containing inactivated *E. rhusiopathiae* and parvovirus and Diluvac Forte® adjuvant (Porcilis® Ery + Parvo, MSD Animal Health) that was administered to the control animals in the efficacy studies.

A vaccine containing live attenuated PRRS virus (Porcilis® PRRS, MSD Animal Health) that was administered concurrently to the pigs in the safety study.

2.2. Leptospira strains

The *Leptospira* strains used for the vaccine preparation were *L. interrogans*, serogroup Canicola, serovar Portland-vere, strain Ca-12-000; *L. interrogans*, serogroup Icterohaemorrhagiae, serovar Copenhageni, strain Ic-02-001; *Leptospira kirschneri*, serogroup Grippotyphosa, serovar Dadas, strain Gr-01-005; *L. interrogans*, serogroup Australis, serovar Bratislava, strain As-05-073; *L. interrogans*, serogroup Pomona, serovar Pomona, strain Po-01-000, *Leptospira santarosai*, serogroup Tarassovi, serovar Gatuni, strain S-1148/02.

The Leptospira challenge strain used was *L. interrogans*, serogroup Pomona, serovar Pomona, strain 02-0162. All strains were obtained at an unknown passage level and all except for strain S-1148/02 were obtained from Dr. C. Bolin, USD, NADC, USA. The Tarassovi strain S-1148/02 was obtained from Queensland Health Science Services, Australia.

After arrival the strains had 2–3 EMJH medium passages after which they were stored in liquid nitrogen. The vaccine strains had 5–7 further medium passages before they were used in the vaccine. After arrival, the Pomona challenge strain was passed through hamsters and after re-isolation and two medium passages stored in liquid nitrogen. Before challenge this strain had two further EMJH medium passages.

2.3. Laboratory safety study

Twenty-six gilts, 16 weeks of age, with undetectable levels of antibodies to *E. rhusiopathiae*, Parvovirus, PRRS virus and *Leptospira* serogroups Canicola, Icterohaemorrhagiae, Australis (Bratislava), Grippotyphosa, Pomona and Tarassovi, were used. A group of 12 gilts was vaccinated with a single dose of 2 ml intramuscularly in the neck with Porcilis® Ery + Parvo + Lepto and was necropsied two weeks later. The vaccine injection site of these pigs was investigated post-mortem for any local tissue reactions or vaccine remnants. A second group of 14 gilts was vaccinated with a standard dose of Porcilis PRRS (left side) and a double dose (4 ml, right side) of Porcilis® Ery + Parvo + Lepto, followed by two single doses of both vaccines with two week intervals between the vaccinations. All pigs were observed daily for local and systemic reactions during 14 days after each vaccination. Rectal temperature was determined on day 1, 2 and 3 before each vaccination, just before vaccination, 4 h after vaccination and then once daily for four days after each vaccination.

2.4. Laboratory efficacy study

2.4.1. Study design

Forty gilts, 20 weeks of age, with undetectable levels of serum antibodies against *Leptospira* serogroups Canicola, Icterohaemorrhagiae, Australis (Bratislava), Grippotyphosa, Pomona and Tarassovi, were used. Twenty gilts were vaccinated twice with Porcilis® Ery + Parvo + Lepto (vaccinates) with a 4-week interval between vaccinations. The other 20 were vaccinated with a commercial batch of Porcilis® Ery + Parvo (controls) using the same vaccination schedule. Twenty gilts (10 vaccinates and 10 controls) were inseminated, after oestrus synchronization, at 41 weeks of age. The other 20 gilts were inseminated, after oestrus synchronization, at 66 weeks of age. The pigs were challenged intravenously (10 ml) and conjunctivally (0.25 ml per eye) with a culture of a virulent strain of *Leptospira* serovar Pomona, strain 02-0162 (10^9 bacteria/ml) 10 weeks after insemination (i.e. 6 or 12 months after the last vaccination). Before challenge one animal was lost due to recurrent locomotory problems, leaving 9 animals for the 12 months challenge control group.

Blood samples were taken for serology, using the microscopic agglutination test (MAT), after vaccination and challenge. Blood and urine samples were taken before and after challenge to isolate the challenge strain.

The pigs were observed regularly for clinical signs, including lethargy, anorexia and abortion, for up to 4 weeks after challenge. Rectal temperature was measured the day before challenge, the

day of challenge (just before) and on days 1, 2, 3, 4, 7 and 10 after challenge.

The pigs were euthanized 4 weeks after challenge. Necropsy was performed and the state of the pregnancy and condition of the foetuses were evaluated. Kidney samples were taken from all pigs and a representative foetus, along with peritoneal fluid, for re-isolation of challenge organisms.

2.4.2. Serology

Blood samples were collected from each pig on the day of vaccination and 4, 6, 12, 31 and 56 weeks after vaccination into Serum Sep. Clot Activator tubes (without anticoagulant, for preparation of serum). Additional blood samples were collected 4 weeks after challenge. The serum samples were stored frozen until analysis. Serogroup specific agglutination titres were determined using the microscopic agglutination (MAT) test as described previously [7].

2.4.3. Isolation of challenge organisms from blood, urine, kidney or peritoneal fluid

To isolate the challenge strain, blood samples were taken, into heparinized tubes, just before challenge, 5 hours after challenge and on days 1, 2, 3, 4, 7 and 10 post-challenge. Urine was sampled just before challenge and on days 14, 17, 21, 24 and 28 post-challenge for re-isolation of challenge strain. At necropsy, a 1–2 g sample was taken from the renal cortex of one kidney of each sow. At necropsy, a kidney sample and peritoneal fluid (0.5 ml) was collected from the smallest viable foetus from each pig.

Blood (heparinized) or urine samples (0.5 ml) were added to 10 ml of liquid EMJH medium containing 200 μg/ml 5-fluorouracil and 1% (v/v) rabbit serum negative for agglutinating antibodies against the 6 different serovars included in the vaccine. Each kidney sample was put into liquid EMJH medium (as described above) and homogenized using an Ultraturrax. A 100-fold dilution of the kidney homogenate was cultured in liquid EMJH medium. Peritoneal fluid (0.5 ml) was inoculated directly into 4.5 ml liquid EMJH medium.

The samples were then incubated at 28–30 °C, and observed fortnightly using dark-field microscopy for at least 8 weeks before negative cultures were discarded. The identity of the isolates was confirmed using the MAT test with specific anti-sera.

2.4.4. Evaluation of pregnancy

Ultrasonography was performed 4 weeks after insemination and just before challenge to confirm pregnancy. After challenge the pigs were observed for abortions by visual inspection and the uterine contents were examined post mortem.

2.4.5. Necropsy

At the end of the study (28 days after challenge) the sows were euthanized humanely using electric stunning followed by exsanguination, and immediately thereafter necropsy. Macroscopic post mortem examination focussed particularly on the lungs, liver, kidneys and spleen. Where macroscopic tissue changes or abnormalities were observed, samples were collected for histological examination. In addition the uterine contents were evaluated for foetal viability, recent foetal death, foetal maceration and mummification. Kidney samples were taken from all gilts and from selected foetuses along with peritoneal fluid for isolation of the challenge organism.

2.5. Field study

An observational-longitudinal field study was conducted on a farm in Portugal with a history of increasing abortion rates associated with *Leptospira* infection.

At the start of the study, all breeding sows and replacement gilts on the farm were vaccinated twice with Porcilis® Ery + Parvo + Lepto with an interval of 4 weeks. Starting six months after the primary vaccination schedule, the animals were re-vaccinated in the second week of every subsequent lactation. New replacement gilts were vaccinated according to the same schedule.

The reproductive performance was monitored and farrowing results and relevant breeding data were collected to determine if vaccination had any effect on the incidence of abortion. The results from the study period were compared to the historical data.

2.6. Statistical analysis

The level of significance α was set at 0.05 and all tests were two sided. Statistical analyses were carried out using the statistical programme SAS V9.1 or higher (SAS Institute Inc. Cary NC, USA).

2.6.1. Rectal temperature

In the laboratory efficacy study, the rectal temperature data over time were statistically evaluated by a linear mixed model ANOVA, using the pre-challenge values as baseline and taking into account the repeated measurement structure of the data [8]. In addition, the effect of vaccination on rectal temperature was evaluated on the peak increase and the Area under the Curve (AUC) of the rectal temperature over time, using for both the pre-challenge data as baseline. AUC was calculated by the linear trapezoidal rule. AUC and the Peak response were statistically evaluated by ANOVA.

2.6.2. Clinical signs: lethargy/anorexia

The duration of clinical signs of lethargy and/or anorexia observed for each animal was analyzed by ANOVA and in addition the number of pigs showing these signs was analyzed using Fisher's exact test.

2.6.3. Bacterial isolation from blood

Bacterial re-isolation data over time in blood, categorized as positive or negative were statistically evaluated by Generalized Estimating Equations (GEE, with binary distribution and the logit as link function), taking into account the repeated measurement structure of the data [9]. In addition the duration of days positive was evaluated by ANOVA.

2.6.4. Foetal death after challenge

At necropsy, foetuses were scored as viable or dead. Dead foetuses were subdivided into recent death, macerated or mummified. The length (cm) of mummified foetuses was measured in order to be able to estimate the age at death using the formula: length (in mm) = $3.25x - 70.59$, where x = days of gestation [10]. Since challenge took place on day 70 of gestation, mummies $\leq$15.7 cm were regarded as having died before challenge (aspecific cause) whereas mummies >15.7 cm were regarded as having died after challenge. The total number of foetuses that died after challenge was calculated by the addition of mummies >15.7 cm + macerated foetuses + recent foetal death. The number of dead foetuses that died after challenge as fraction of the total number of foetuses from that litter was analyzed by Poisson regression, with the effect of litter taken into account (GEE) [9].

2.6.5. Evaluation of field study

Abortion rate per year was analyzed by logistic regression using a log linear model to estimate the relative risk on an abortion [9,11]. A year was defined as the period of 1 December in any calendar year to 30 November in the subsequent calendar year e.g. 2012 was from 1 December 2011 to 30 November 2012.

Table 1
Reproductive performance of pregnant gilts challenged with serovar Pomona at 10 weeks of gestation and 6 or 12 months after vaccination.

Group	Time after vaccination	*n*	Number of foetuses						Logistic regression GEE
			Total	Total alive	Total dead	Dead before[a]	Dead after[b]	% Dead after challenge	
vac	6 (months)	10	128	121	7	4	3	3	$p = 0.0003$
con	6 (months)	10	150	104	46	15	31	21	
vac	12 (months)	10	145	138	7	4	3	2	$p = 0.0076$
con	12 (months)	9	128	83	45	11	34	27	

[a] Mummies <15.7 cm length.
[b] Mummies/foetuses >15.7 cm length.

3. Results

3.1. Laboratory safety study

No vaccine-related rectal temperature increase or other systemic reactions were observed after vaccination (data not shown).

No local reactions were observed following vaccination, except one gilt, which had a small local reaction (2 cm diameter) for 5 days after the third vaccination. At necropsy injection site reactions, characterized by pale tissue discoloration, were observed macroscopically in two animals. Histologically, these reactions appeared to consist of limited granulomatous inflammatory tissue.

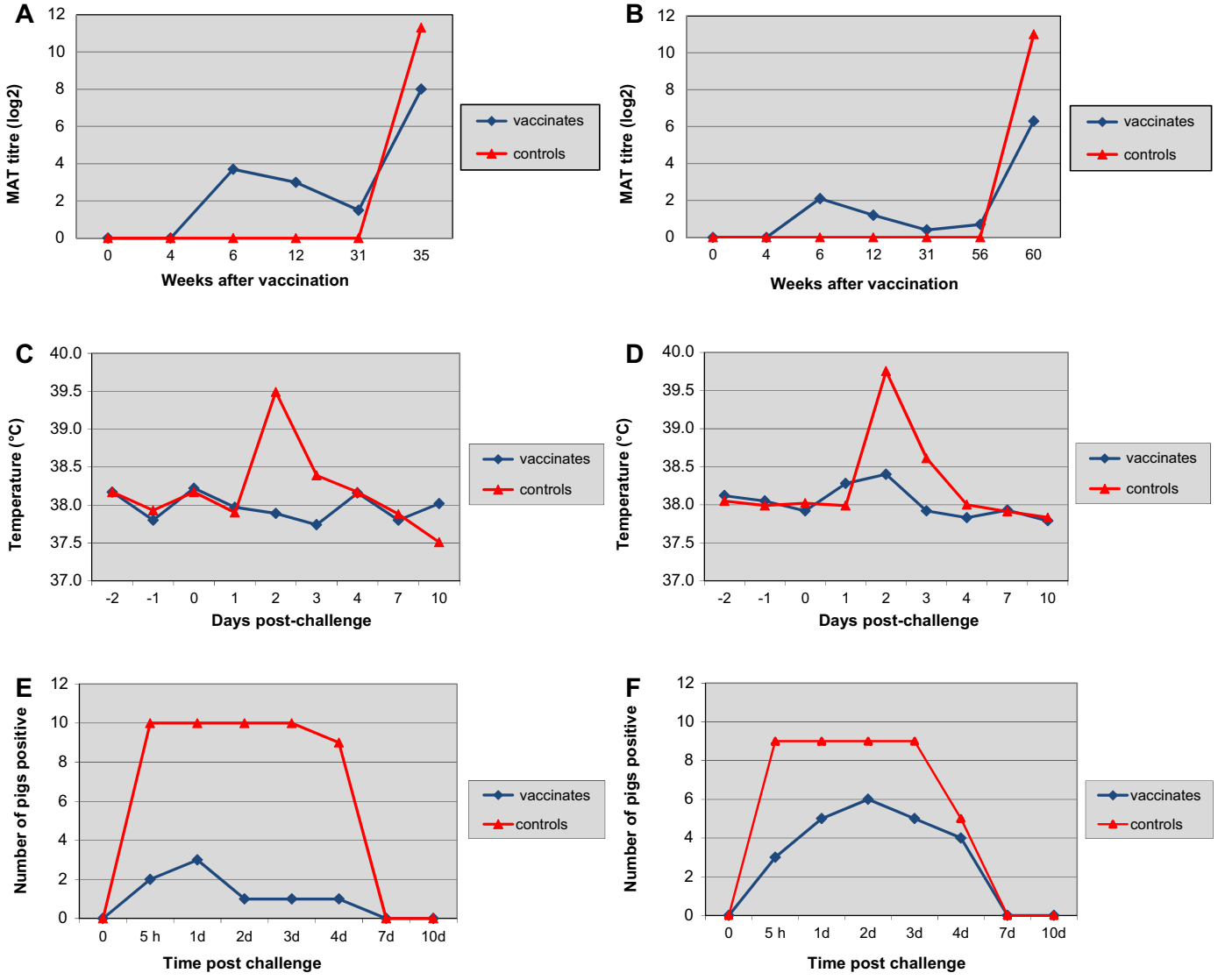

Fig. 1. Time-course of average *Leptospira* serovar Pomona specific MAT antibody titres after vaccination and challenge (A and B) and time-course of average rectal temperature (C and D) and leptospiraemia (E and F) after challenge with *Leptospira* serovar Pomona. Pigs were vaccinated with Porcilis® Ery + Parvo + Lepto (vaccinates) or with Porcilis® Ery + Parvo (controls) at 20 and 24w of age. Pigs were inseminated at 41 or 66 weeks of age and challenged with *Leptospira* serovar Pomona at 51 weeks of age (A, C and E) or 76 weeks of age (B, D and F) corresponding to 6 and 12 months after last vaccination. The difference in temperature peak response as well as the AUC were significantly different ($p < 0.05$, ANOVA) between vaccinates and controls after both the 6 (B) and 12 (C) months challenge. The days being leptospiraemic was significantly different ($p < 0.05$, ANOVA) between vaccinates and controls after both the 6 (E) and 12 (F) months challenge.

3.2. Laboratory efficacy study

3.2.1. Serology

After vaccination, approximately half of the vaccinated gilts developed detectable MAT titres against serovar Pomona. The average titres peaked 2 weeks after second vaccination (range 0–8 $\log_2$) after which they declined and many vaccinates were seronegative by the day of challenge (Fig. 1A and B). The control animals remained seronegative until challenge. All pigs showed a strong increase in serum antibody titre after challenge, at 6 or 12 months. There was a more marked increase in serum antibody titres in the controls than in the vaccinates.

3.2.2. Rectal temperature

An increase in average rectal temperature was seen in the controls at day 2 after challenge, at 6 or 12 months (Fig. 1C and D). There was a statistically significant difference in the peak rectal temperature after challenge between the vaccinates and controls; $p = 0.0002$ at 6 months and $p < 0.0001$ at 12 months. The area under the rectal temperature curve (AUC) was significantly lower in the vaccinates compared to the controls; $p = 0.0020$ and $p = 0.0075$, respectively.

3.2.3. Clinical signs

Lethargy and/or anorexia was observed in all of the controls from day 2 after challenge, whereas most of the vaccinates did not show clinical signs (data not shown). The duration of clinical signs was significantly shorter in the vaccinates than in the controls after both the 6 and 12 months challenge; $p = 0.0002$ and $p = 0.0110$, respectively. The incidence of lethargy was significantly lower in the vaccinates than in the controls after the 6 months challenge ($p < 0.0001$) but not after the 12 months challenge ($p = 0.1789$).

3.2.4. Re-isolation of challenge organisms from blood and urine

After challenge all of the controls became infected and were leptospiraemic for 3–4 days (Fig. 1E and F). After the 6-month challenge, both the number of pigs infected ($p = 0.0031$) as well as the number of days positive ($p < 0.0001$) were significantly different between the vaccinates and the controls. After the 12-month challenge, the number of pigs infected did not differ significantly between groups ($p = 0.4370$) but the vaccinates were culture positive for significantly fewer days than the controls ($p = 0.0048$).

There were no positive urine cultures following challenge in either group.

3.2.5. Reproductive performance

No macroscopic tissue changes were found outside the uterus at necropsy. There was more foetal death, autolysis and/or mummification in the controls than in the vaccinates (Table 1). The number of dead foetuses after challenge as a fraction of litter size was significantly lower in the vaccinates than in the controls following challenge at 6 ($p = 0.0003$) or 12 months $p = 0.0076$.

All kidney cultures (sow and foetus) were *Leptospira* negative except for one control sow from the 12-month challenge group. One foetal peritoneal fluid sample from the controls at the 6-month challenge and two samples from the controls at the 12-month challenge were Pomona culture positive.

3.3. Field study results

3.3.1. Field study results pre-vaccination

Abortions had a seasonal pattern peaking in the four winter months. They first appeared in 2011 when the abortion rate rose to 4% in the four winter months (and 2.3% over the whole year period). No action was taken at this time because the abortion rate was not alarming. The situation deteriorated in 2012 when the abortion rate reached 12.6% in the winter months (and 4.4% over the whole year period). Abortions were late in gestation and no other clinical signs were observed at that time on the farm. *Leptospira* was diagnosed from aborted foetal material sent to a regional veterinary diagnostic laboratory. In December 2011, aborted foetuses were PCR positive for *Leptospira* and negative on PCR for PRRSV and in January 2012 aborted foetal material was again PCR positive for *Leptospira* and negative on PCR for Toxoplasma. The former results were confirmed by another diagnostic laboratory using PCR for *Leptospira* and serology for PRRSV. In March 2012, blood samples were taken at random from 36 pigs and sera tested in the MAT test. High MAT titres (up to 9 $\log_2$) against serogroup Pomona were found in four animals whereas titres against other serogroups (including Canicola, Icterohaemorrhagiae, Grippotyphosa, Australis and Tarassovi) were virtually absent, indicating a recent *Leptospira* serovar Pomona infection (Table 2).

Table 2
Serological results of Portuguese farm (36 sera tested).

MAT titre	Frequency of MAT titre					
$\log_2$	Canicola	Ictero	Bratislava	Grippo	Pomona	Tarassovi
≤2	34	36	36	35	31	36
3	**2**	0	0	0	**1**	0
4	0	0	0	**1**	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	**2**	0
8	0	0	0	0	0	0
9	0	0	0	0	**2**	0

Because of the abortion storm and the Pomona positive and PRRSV negative diagnosis; this farm was selected for a field study using Porcilis® Ery + Parvo + Lepto. At the start of the study (Oct-Nov 2012), all breeding sows and replacement gilts on the farm were vaccinated with Porcilis® Ery + Parvo + Lepto.

3.3.2. Field study results post-vaccination

After vaccination, the abortion rate reduced rapidly from 12.6% in winter months of 2012 (December 2011 to March 2012) to 0.5% in winter months of 2013, a statistically significant decrease of 96%. The total number of abortions on the farm decreased from 55 in 2012 to 6 in 2013 (Table 3 and Fig. 2). The number of abortions on the farm remained stable after the vaccination programme was started and was 0.6% in the period December 2013 to April 2014. The results of logistic regression are summarized in Table 4. There was no statistically significant difference in the abortion rate in the years 2010, 2013 and 2014 but the abortion rates in these years were significantly lower than in 2011 and 2012.

Table 3
Reproduction data Portuguese field trial.

Period	01.12.09 30.11.10	01.12.10 30.11.11	01.12.11 30.11.12	01.12.12 30.11.13	01.12.13 30.04.14
Farrows (*N*)	1244	1275	1253	1206	489
Abortions (*N*)	11	29	55	6	3
% Abortions	0.9	2.3	4.4	0.5	0.6
Total born (avg)	13.3	14.1	14.0	15.3	15.0
Born alive (avg)	12.3	12.9	13.0	14.4	14.0
Wean (avg)	10.9	11.5	11.7	12.8	12.5
% Stilbirths	7.5	8.5	7.1	5.5	6.7
% Wean mortality	11.4	10.9	10.0	11.1	10.7
Sows total	1338	1381	1398	1336	476
Pregnant (*N*)	1267	1305	1259	1247	441
% Pregnant	94.7	94.5	90.1	93.3	92.6

Table 4
Summary statistical results abortion rate.

Comparison periods	Relative risk on abortion	Lower limit 95%CI	Upper limit 95%CI	*p*-Value (Type 3)
2014[b] vs 2010[a]	0.70	0.19	2.48	0.5632
2013 vs 2010	0.56	0.21	1.52	0.2484
2014 vs 2013	1.23	0.31	4.91	0.7703
2010 vs 2011	0.39	0.20	0.79	0.0051
2013 vs 2011	0.22	0.09	0.53	0.0001
2014 vs 2011	0.27	0.08	0.90	0.0107
2010 vs 2012	0.21	0.11	0.40	<0.0001
2013 vs 2012	0.12	0.05	0.27	<0.0001
2014 vs 2012	0.15	0.05	0.46	<0.0001
2011 vs 2012	0.53	0.34	0.82	0.0038

[a] Period 2010, 2011, 2012 and 2013 is from 1st Dec.–30 Nov. of the subsequent year.
[b] Period 2014 is from 1st Dec. 2013–30 Apr. 2014.

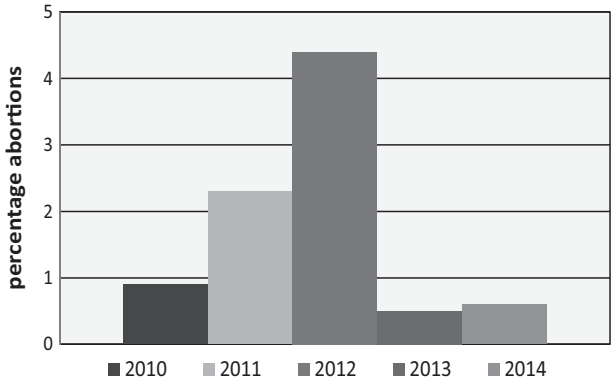

Fig. 2. Time-course of per cent abortions on a Portuguese farm before and after vaccination with Porcilis® Ery + Parvo + Lepto; vaccination was started in October 2012. There was a statistically significant reduction in abortion rate after vaccination ($p < 0.05$, logistic regression analysis) compared to previous years.

4. Discussion

The present study supports the safety of the new Porcilis® Ery + Parvo + Lepto vaccine in gilts. An increase in rectal temperature, commonly observed after vaccination with most vaccines, was absent in this study. In addition, no other systemic reactions were observed and only one transient (5 days), small local reaction (2 cm) was found after the third vaccination. Necropsy of the vaccine injection sites, 2 weeks after vaccination, revealed two macroscopically visible local reactions, characterized by pale tissue discoloration, which were composed of limited granulomatous inflammatory tissue, which is a normal reaction that can be expected after vaccination with a vaccine containing multiple bacterial antigens and adjuvant.

Since Pomona is the most notorious serovar associated with foetal death [6], the present study focused on efficacy against this serovar in a controlled laboratory challenge study as well as under field conditions. The laboratory challenge study demonstrated that serovar Pomona was capable of inducing clinical signs (fever and lethargy), leptospiraemia and foetal death in up to 27% of the control group. Leptospiruria was not observed in this study; all urine cultures remained negative. This is in contrast to the results of Hodges et al. [4] and Whyte et al. [6] who found urinary shedding of Pomona after challenge as evidenced by *Leptospira* positive urine cultures.

Vaccination resulted in a significant reduction in clinical signs, leptospiraemia and, most importantly, foetal death. The fact that serovar Pomona was isolated from peritoneal fluid samples of three control foetuses underlines the relation between this serovar and reproductive failure. The fact that only three samples were positive, confirms the difficulties associated with the diagnosis of leptospirosis. Apparently animals can be infected but be culture negative. Likewise, after laboratory challenge the bacterium can be isolated from blood for up to 4 days after which the animals become culture negative, including urine culture, but the *Leptospira* are still present in the animal, causing foetal death.

Vaccination induced only low to moderate Pomona specific MAT antibody titres and many vaccinates remained seronegative. Despite this, these vaccinated animals were protected against laboratory challenge. It is possible that very low concentrations (below the lower limit of quantification of the assay) of serum agglutinating antibodies may protect against infection. This is in line with several earlier studies [12–14] which showed that sera from vaccinated animals or humans with low or undetectable concentrations of agglutinating antibodies afforded protection in the passive hamster protection test.

After challenge high antibody titres were induced, especially in the control animals which had a more marked increase. This is in line with the results of Whyte et al. [6] and may be a reflection of vaccine efficacy and rate of antigen exposure in the control animals compared to the vaccinated animals.

After these promising laboratory results the vaccine was tested under field conditions on a Portuguese farm with increasing abortion rates associated with *Leptospira* infection. PCR on aborted material was tested *Leptospira* positive by two independent laboratories and subsequent serological testing demonstrated Pomona specific antibodies whereas other serovars tested virtually negative.

The study was designed as an observational-longitudinal field study. After vaccination a rapid, and statistically significant, reduction in abortion rate was manifested, from 12.6% in the winter of 2012 (December 2011 to March 2012) down to 0.5% in the winter of 2013 (December 2012 to March 2013), a decrease of 96% in the abortion rate compared to the previous year.

In conclusion, the present studies demonstrate that the octavalent vaccine Porcilis® Ery + Parvo + Lepto can be safely used in gilts and sows and induces statistically significant protection that lasts for at least one year against serovar Pomona induced clinical signs, leptospiraemia and foetal death. Protection against Pomona associated reproductive failure was confirmed under field conditions where a significant reduction in abortion rate was observed.

Conflict of interest

All authors are employed by MSD.

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