(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 November 2024 (21.11.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/235705 A1

(51) International Patent Classification:
A61K 39/02 (2006.01) *A61K 39/23* (2006.01)
A61P 31/04 (2006.01)

(21) International Application Number:
PCT/EP2024/062431

(22) International Filing Date:
06 May 2024 (06.05.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
23173014.4 12 May 2023 (12.05.2023) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*

WO 2024/235705 A1

(54) **Title:** A COMBINATION VACCINE AGAINST ERYSIPELOTHRIX RHUSIOPATHIAE, PORCINE PARVO VIRUS AND LEPTOSPIRA BACTERIUM

(57) **Abstract:** The invention pertains to a combination of a first vaccine comprising a non-replicating immunogen of *Erysipelothrix rhusiopathiae* and a non-replicating immunogen of porcine parvo virus, and a second vaccine comprising a non-replicating immunogen of a Leptospira bacterium, for use in prophylactically treating a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium, by associated separate injection of the first vaccine and the second vaccine into the dermis of the pig at a first and a second injection site of that pig respectively.

A COMBINATION VACCINE AGAINST ERYSIPELOTHRIX RHUSIOPATHIAE, PORCINE PARVO VIRUS AND LEPTOSPIRA BACTERIUM

GENERAL FIELD OF THE INVENTION

The invention pertains to the prophylactic treatment of a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium by vaccination.

BACKGROUND OF THE INVENTION

Pigs are prone to many infections and disorders arising from such infections. For example, erysipelas is an infectious disease caused by *Erysipelothrix rhusiopathiae* ("ery") and is one of the oldest recognized diseases that affect growing and adult swine. Up to 50% of pigs in intensive swine production areas are considered to be colonized with *E rhusiopathiae*. The organism commonly resides in the tonsillar tissue. Typically, healthy carriers shed the organism in their feces or oronasal secretions and are an important source of infection for other pigs. Infection is by ingestion of contaminated feed, water, or feces and through skin abrasions. When ingested, the organism can survive passage through the hostile environment of the stomach and intestines and may remain viable in the feces for several months. On farms where the organism is endemic, pigs are exposed naturally to *E rhusiopathiae* when they are young. Maternal-derived antibodies provide passive immunity and suppress clinical disease. Older pigs tend to develop protective active immunity as a result of exposure to the organism, which does not necessarily lead to clinical disease. Recovered pigs and chronically infected pigs may become carriers of *E rhusiopathiae*. Healthy swine also may be asymptomatic carriers.

Vaccination against *E rhusiopathiae* is very effective in controlling disease outbreaks on swine farms and should be encouraged. It may not be as effective in preventing chronic arthritis, however. Cessation of vaccination on some farms has been linked to disease

outbreaks. Injectable bacterins and attenuated, live vaccines delivered via the water are available and provide extended duration of immunity. Optimal timing of vaccination may vary from farm to farm. When *E rhusiopathiae* is endemic in the production environment, vaccination should precede anticipated outbreaks. Susceptible pigs may be vaccinated before weaning, at weaning, or several weeks after weaning. Male and female swine selected for addition to the breeding herd should be vaccinated with a booster 3–5 weeks later. Thereafter, breeding stock should be vaccinated twice yearly with a follow up vaccination. Vaccination is however not always straight forward for arriving at protection. Vaccination failures may occur in some herds due to management stresses that compromise the immune system of vaccinated pigs. The use of live vaccines may also lead to clinical disease, particularly chronic erysipelas. Antigenic differences between serotypes in vaccines and serotypes circulating on farms could also result in incomplete immunity and disease outbreaks, but this is a rare event because there is thought to be good cross-protection among the major *E rhusiopathiae* strains infecting pigs.

Also, porcine parvovirus infection (PPV, or “parvo”) is a common pathogen of swine. It is an important cause of infectious infertility. PPV is a robust virus that multiplies normally in the intestine of the pig without causing clinical signs and is ubiquitous in pig populations world-wide. PPV is one of the organisms listed as responsible within the Stillbirths Mummification Embryonic Death and Infertility (SMEDI) syndrome. In larger herds it is almost certain to be present and is an infection one has to live with and manage, rather than try to eliminate. In smaller herds with a previously PPV positive pig, it may or may not have died out. Most viruses do not survive outside the host for any great length of time, PPV is unusual in that it can persist in the environment for many months and it is resistant to most disinfectants, and the most likely reason as to why is widespread and difficult to eliminate. PPV is most often transmitted either by mouth or through the snout, passing into the intestine where it multiplies and subsequently passed out in faeces. When a pig becomes infected for the first time there are no clinical signs. Boars may also infect a sow at the time of mating via their semen.

PPV cannot be eliminated from a herd so management and prevention of acute cases should be the aim. Routine vaccination of gilts and boars prior to entering the breeding herd plus yearly follow-up vaccinations of all pigs should be sufficient to ensure the herd is protected. It is known that if an infected breeding female has been vaccinated against PPV at some time in the past, then when exposure to PPV takes place, there is rapid re-

stimulation of the immune system (within 5-7 days). This is sufficient to prevent disease and to stimulate a (semi-)permanent immunity.

Next, leptospirosis is a contagious disease of swine and many other animals (including humans) and is caused by infection with any one of a large group of Leptospira bacteria ("lepto"). Leptospira are motile spirochetes, 6-12 microns long, and 0.1 micron in diameter. They usually are hooked on both ends and can be stained by Giemsa stain or, in tissue, by silver stains. In the laboratory they often are studied under darkfield microscopy. In laboratory media they are difficult to culture and grow very slowly (12-26 weeks). Many pathogenic strains can survive in the environment for long periods of time under moist conditions with a slightly alkaline pH. Most cannot withstand desiccation and are destroyed by common disinfectants.

Although there are generalities of leptospirosis in swine, details of infection with different serovars may vary. Swine are susceptible to many different serovars. Classification for Leptospira is based largely on DNA relatedness to known reference strains but universal agreement on taxonomy of the genus remains elusive. Under the most current classification, the family Leptospiraceae contains eight pathogenic species of which three are of most importance to swine: *Leptospira interrogans* (serovars *pomona, icterohaemorrhagiae, canicola*, and *bratislava*), *Leptospira borgpetersenii* (serovars *sejroe* and *tarassovi*) and *Leptospira kirschneri* (serovar *grippotyphosa*). Serovars *pomona* and *bratislava* are uniquely adapted to swine, others are maintained in other species but sometimes infect swine. *Leptospira bratislava* is reported to be the most common strain in swine, although the role of this serotype as a cause of disease is debated.

Control of leptospira is usually attempted by prevention of exposure, immunization through vaccination, and/or the use of antibiotics. Prevention of exposure is difficult to achieve because so many species can act as carriers of leptospires. These include infected swine, rodents (especially mice and rats) and many kinds of wildlife. Once leptospires are introduced into a favorable wet environment, they often persist there as a source of infection. Nonetheless, leptospirosis can be effectively controlled (perhaps even eradicated) from swine populations raised under confinement conditions using a combination of medication, vaccination, vector control, and providing a treated or non-contaminated drinking water source. Immunization through use of bacterins, widely practiced in breeding herds, usually will reduce the prevalence of infection and

abortions. The bacterin must be appropriate for the serovar of Leptospira causing the disease. Many bacterins are thus multivalent.

PRRS virus was first reported in 1987 in North America and Central Europe. PRRS virus is a small, enveloped RNA virus. It contains a single-stranded, positive-sense, RNA genome with a size of approximately 15 kilobases. The genome contains nine open reading frames. The virus is a member of the genus Arterivirus, family Arteriviridae, order Nidovirales. The two prototype strains of PRRSV are the North American strain, VR-2332, and the European strain, the Lelystad virus (LV). The European and North American PRRSV strains cause similar clinical symptoms. In the early 2000s a highly pathogenic strain of the North American genotype emerged in China. This strain, HP-PRRSV, is more virulent than all other strains, and causes great losses in Asian countries worldwide. For any PRRS virus, subclinical infections are common, with clinical signs occurring only sporadically in a herd. Clinical signs include reproductive failure in sows such as abortions and giving birth to stillborn or mummified fetuses, and cyanosis of the ear and vulva. In neonatal pigs, the disease causes respiratory distress, with increased susceptibility to respiratory infections such as Glässer's disease.

Vaccine compositions against all of the above identified pathogens are commonly known.

OBJECT OF THE INVENTION

It is an object of the invention to provide a new method for convenient, safe and effective vaccination against one or more of the swine pathogens as mentioned here above.

SUMMARY OF THE INVENTION

In order to meet the object of the invention, it has been found that a combination of a first vaccine comprising a non-replicating immunogen of *Erysipelothrix*

rhusiopathiae and a non-replicating immunogen of porcine parvo virus, and a second vaccine comprising a non-replicating immunogen of a Leptospira bacterium, can be used in the prophylactic treatment a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium, by associated separate injection of the first vaccine and the second vaccine into the dermis of the pig at a first and a second injection site of that pig respectively. At typical intradermal vaccination volumes below 1 ml, typically around 0.2 ml, if the first and second injection sites are more than 1.5 - 2 cm's apart, the two vaccines will not mix underneath the skin and local reactions of each vaccine can be distinguished from the other. This means, that in line with the EMA Guideline on the requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs), 18 July 2013 (EMA/CVMP/IWP/594618/2010), which guideline defines "Separate sites" as application sites "*sufficiently distant from each other to prevent the possibility of mixing of the products and to allow local reactions to each product to be distinguished from each other*", the injection sites can be regarded as "separate" in the sense of the present invention. With intradermal vaccination, any distance between the first and second injection site above 1.5, preferably above 2 cm, will thus lead to the same result of preventing negative interference regarding the safety and/or efficacy of the associated vaccination of the two vaccines, due to the lack of mixing of these two vaccines after administration.

This way, safe and effective vaccination of a swine can take place in a convenient way. Although separate and even combination vaccines are available for swine, it is not a given that a new site of administration will lead to a safe and effective vaccine, even if the antigens are the same.

The World Health Organization (WHO) for example has published an e-learning course called "Vaccine Safety Basics" (https://apps.who.int/iris/handle/10665/340576), in which course on page 53 it is reported that "*The route of administration is the path by which a vaccine (or drug) is brought into contact with the body. This is a critical factor for success of the immunization. A substance must be transported from the site of entry to the part of the body where its action is desired to take place. Using the body's transport mechanisms for this purpose, however, is not trivial.*"

In this respect the California Department of Health Services' Immunization Branch has published guidelines for correct immunization

(https://www.cdc.gov/vaccines/pubs/pinkbook/vac-admin.html). With regard to the administration site it is stated on page 81, second full paragraph titled "Route and Site for Vaccination", that "*The recommended route and site for each vaccine are based on clinical trials, practical experience and theoretical considerations. There are five routes used to administer vaccines. Deviation from the recommended route may reduce vaccine efficacy or increase local adverse reactions.*"

All in all, it is commonly known that vaccination at a particular site is not straightforward and requires experimentation to determine if safety and efficacy can be arrived at, and if so, if the level is adequate for commercial use.

Next to a combination of a first and second vaccine for a use as described here above, the invention also pertains to a kit-of-parts comprising a first vaccine comprising a non-replicating immunogen of *Erysipelothrix rhusiopathiae* and a non-replicating immunogen of porcine parvo virus, and a second vaccine comprising a non-replicating immunogen of a Leptospira bacterium, for use in prophylactically treating a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium, by associated separate injection of the first vaccine and the second vaccine into the dermis of the pig at a first and a second injection site of that pig respectively.

The invention also pertains to a method of prophylactically treating a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium, by associated separate injection of a first vaccine comprising a non-replicating immunogen of *Erysipelothrix rhusiopathiae* and a non-replicating immunogen of porcine parvo virus, and a second vaccine comprising a non-replicating immunogen of a Leptospira bacterium into the dermis of the pig.

Lastly, the invention is embodied in the use of a non-replicating immunogen of *Erysipelothrix rhusiopathiae* and a non-replicating immunogen of porcine parvo virus to manufacture a first vaccine, and the use of a non-replicating immunogen of a Leptospira bacterium to manufacture a second vaccine, the combination of both these vaccines for prophylactically treating a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium, by associated separate injection of the first vaccine and the second vaccine into the dermis of the pig at a first and a second injection site of that pig respectively.

DEFINITIONS

A *vaccine* is a pharmaceutical composition that is safe to administer to a subject animal, and is able to induce protective immunity in that animal against a pathogenic micro-organism, i.e. to induce a successful prophylactic treatment as defined here below.

Administration of a vaccine into the dermis means the vaccine is at least partly deposited in the dermis. The World health Organization in its August 27, 2009 paper called "Intradermal Delivery of Vaccines; A review of the literature and the potential for development for use in low- and middle-income countries" indicates that "needle-less" vaccination does not necessarily mean "intradermal" vaccination (see Table 1, Page 3 of the review). Only when a needle-less device is "configured for intradermal vaccination", then a vaccine may indeed be delivered into the dermis. Otherwise it may be delivered subcutaneous or intramuscularly. Several devices are commercially available for intradermal vaccination, for example the IDAL® vaccinator (MSD Animal Health), the Pulse 50 MicroDose (Pulse Needle Free Systems), or other devices as described in *Vaccine*, 2012 Jan 11;30(3):523-38 (see in particular Table 1, page 525: " An overview of different devices for liquid and solid formulation administration")

A *combination of a first and second vaccine* is a set of vaccines, wherein the first vaccine is individually distinct from the second vaccine, but wherein the two vaccines are used in conjunction for administration to the same subject in one concerted treatment.

Non-replicating immunogen of a pathogen is any substance or compound corresponding to the pathogen, other than the live replicating pathogen as a whole (either in wild type of attenuated form), against which pathogen an immunological response is to be elicited, such that the corresponding virulent pathogen or one or more of its virulence factors will be recognized by the host's immune system as a result of this immune response and are ultimately at least partly neutralized. Typical examples of non-replicating immunogens are killed whole pathogens (which term includes these pathogens in lysed form) and subunits of these pathogens such as capsid proteins, surface expressed molecules (for example recombinantly expressed proteins or

lipopolysaccharides) and excreted molecules such as toxins.

A *bacterin* is a suspension of killed bacteria, either as whole cells, partly lysed or completely lysed (such as for example by homogenisation, French pressing, or a combination of two or more lysing methods).

A *live attenuated* pathogen is a viable, replication competent form of the pathogen having reduced virulence. The process of attenuation takes an infectious pathogen and alters it so that it becomes harmless or less virulent, typically by either multiple passages of the pathogen through cell systems or by genetically modifying the pathogen.

Prophylactic treatment against an infection with a pathogen is aiding in preventing, ameliorating or curing an infection with that pathogen or a disorder arising from that infection, resulting from a post treatment challenge with the pathogenic pathogen, in particular to reduce its load in the host after such challenge or to aid in preventing or ameliorating one or more clinical manifestations resulting from the post treatment infection with the pathogen.

A *pig* is an animal belonging to the family of Suidae, a family of artiodactyl mammals which are commonly called pigs, hogs or boars. Eight-teen extant species are currently recognized (or nineteen counting domestic pigs and wild boars separately), classified into between four and eight genera. Within this family, the genus Sus includes the domestic pig, *Sus scrofa domesticus* or *Sus domesticus*.

Associated separate administration of vaccines, also referred to as concurrent administration, is the administration of these vaccines separately, thus not mixed before administration, to the target animal, but within a time frame such that immunological interference is expected to occur, typically within 24 hours. A typical example of associated use is the simultaneous administration at separate application sites in the target animal, or separated in time by at most 1-24 hours.

Simultaneous administration of vaccines means the administration at exactly the same time or at least within a time frame of 1 hour, preferably within 55, 50, 45, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 minutes or even a time frame of 1 minute, such as at the same time exactly

Single dose administration of a vaccine for use in prophylactically treatment means that in order to arrive at protective immunity, the vaccination does not need to be boosted with a second administration of the vaccine.

A *prime and boost vaccination* scheme means that in order to arrive at protective immunity, a first vaccination is boosted with a second administration of the vaccine. Typically, the prime vaccination is boosted within 6 weeks, commonly within 5 or even 4 weeks from this first vaccination, and after the second (boost) vaccination adequate protective immunity is typically obtained.

FURTHER EMBODIMENTS OF THE INVENTION

In a first further embodiment of the invention, the first and second injection sites are at most 4 cm apart from each other, preferably at most 3 cm apart from each other, even more preferably less than 2.5 cm such as 2 cm. It was found that this way pigs can be vaccinated more conveniently, in particular by using for example the IDAL® 3G Twin device (available from MSD Animal Health, Boxmeer, The Netherlands; distance between administration sites is 2.8 cm) having two barrels, holding separate vaccines, for associated intradermal injection of the two vaccines at the same time.

Further preferred is that the associated separate injection of the first vaccine and the second vaccine occur simultaneously.

In another preferred embodiment of the combination of a first and second vaccine for use according to the invention, the first and second vaccines are given in a prime and boost vaccination scheme, wherein both vaccines are injected at the prime vaccination, and both vaccines are injected at the boost vaccination.

In another embodiment, at least the first vaccine comprises an adjuvant (as is commonly known, leptospira vaccines do not necessarily need an adjuvant to arrive at high levels of protection), but preferably both the first and second vaccine comprises an adjuvant, for example an oil in water adjuvant. Typical adjuvants for use with the invention are oil-in-water adjuvants, such as emulsions of mineral oil in water, or mineral

oil and vitamin-E-acetate in water. These adjuvants can be stabilised with emulsifiers such as polysorbate.

In again another embodiment of the first and second vaccine for use according to the invention, the non-replicating immunogen of *Erysipelothrix rhusiopathiae* is an *Erysipelothrix rhusiopathiae* bacterin. In again another embodiment of the first and second vaccine for use according to the invention the non-replicating immunogen of porcine parvo virus is an inactivated porcine parvo virus. In again another embodiment of the first and second vaccine for use according to the invention the non-replicating immunogen of a Leptospira bacterium is a *Leptospira interrogans* bacterin. Preferably, the second vaccine additionally comprises a *Leptospira kirschneri* and/or a *Leptospira santarosai* bacterin.

In again another embodiment of the first and second vaccine for use according to the invention, the non-replicating immunogen of *Erysipelothrix rhusiopathiae* is an *Erysipelothrix rhusiopathiae* bacterin, the non-replicating immunogen of porcine parvo virus is an inactivated porcine parvo virus, and the non-replicating immunogen of a Leptospira bacterium is a *Leptospira interrogans* bacterin. Preferably, the second vaccine additionally comprises a *Leptospira kirschneri* and/or a *Leptospira santarosai* bacterin.

Advantageously, it was found that the combination of the first and the second vaccine for use according to any of the preceding claims, can also be used in conjunction with a third vaccine that is injected to the pig, the third vaccine comprising a live attenuated PRRS virus, and arrive at a safe and effective combination vaccination against all four pathogens, thus including protection against an infection with PRRS virus. Preferably, in the prophylactic treatment, the pig receives a prime vaccination with the first and second vaccine, and a boost vaccination with the first and second vaccine wherein at the boost vaccination (i.e simultaneous with or at least within 24 hours of this boost vaccination) the third vaccine is injected as a single dose vaccine. In a further embodiment the third vaccine (i.e. the PRRS vaccine) is mixed with the second vaccine (i.e. the Leptospira vaccine) for the boost vaccination.

In an embodiment, in the combination of the first and the second vaccine for use according to the invention, both the first and second vaccine are injected by a jet stream of the respective vaccines using a needle-less device, wherein the jet stream penetrates

the skin of the pig to reach the dermis. The third vaccine is advantageously also injected by a jet stream using a needle-less device.

The invention will now be explained in more detail using the following specific examples.

EXAMPLES

Example 1 is a first trial of an Ery-Parvo-Lepto combination vaccine
Example 2 is a second trial of an Ery-Parvo-Lepto combination vaccine
Example 3 is a third trial of an Ery-Parvo-Lepto combination vaccine

Example 1

Example 1 is a first trial of an Ery-Parvo-Lepto combination vaccine. For this the existing vaccine Porcilis® EPL was used (comprising *E. rhusiopathiae*, serotype 2 bacterin, inactivated porcine parvovirus, and various leptospira bacterins, in this case *L. interrogans* serogroup Icterohaemorrhagiae, *L. interrogans* serogroup Australis serovar Bratislava, *L. kirschneri* serogroup Grippotyphosa, *L. interrogans* serogroup Pomona serovar Pomona and lastly *L. santarosai* serogroup Tarassovi), which is known to be a safe and efficacious vaccine for use in swine, against ery, parvo and lepto infections by intramuscular vaccination. The antigens are all inactivated pathogens. Aim was to see whether the vaccine could also be applied intradermally and still meet predetermined safety and efficacy requirements. The device chosen for the intradermal delivery was the IDAL® 3G Twin device (the twin IDAL). Since the volume per administration is substantially less than what is recommended or Porcilis EPL, namely 0.2 ml instead of 2 ml, the amount of antigens of Porcilis EPL needed to be lowered (to 40 and 25%; see below) to be able and fitted into the smaller volume of the aqueous phase of the formulations.

Various different adjuvants were used in the test, namely the proprietary adjuvant Diluvac Forte (DF; MSD Animal Health, Boxmeer, The Netherlands), µDiluvac forte (µDF; same as Diluvac but with less surfactant and no mineral oil), and an experimental squalane, vitamin E-acetate and silica containing adjuvant (see i.a. WO 2021/048338), here denoted as SVEA. In the two Diluvac adjuvantia 40% of the antigen amount as present in Porcilis EPL could be formulated. In the squalene based adjuvant this was

only 25%.

For the experiment healthy 12-week-old pigs, seronegative for PRRS and Ery, were used. The pigs were divided into 4 groups. The vaccine groups consisted of 10 animals and the control group of 5. Group 1 was vaccinated twice (in a prime-boost scheme) intradermally (ID) with EPL-ID-40%-DF with Porcilis PRRS administered non-mixed (as a separate vaccine) during the booster vaccination; Group 2 was also vaccinated twice (in a prime-boost scheme) ID, but in this group with EPL-ID-40%-µDF with Porcilis PRRS administered non-mixed during the booster vaccination. Group 3 was vaccinated twice ID with EPL-ID-25%-SVEA with Porcilis PRRS administered non-mixed during the booster vaccination. Group 4 was left unvaccinated. All vaccinations were administered intradermally (0.2ml) in the neck (prime right side, boost left side) using the IDAL device and a 4-week interval between the vaccinations.

After vaccination, groups 1, 2 and 3 were observed for local injection site reactions. Three weeks after the last vaccination, groups 1 and 4 were challenged intradermally (0.1ml), each of these groups in the right and left side of the chest with *E rushiopathiae* serotype 1 and serotype 2 challenge strains, respectively. Starting 2 days before challenge the animals were monitored for clinical signs and rectal temperature for 10 days. At each day of vaccination and at day of challenge blood was collected for Ery and Parvo serological examination.

Group 1 showed Ery antibody responses whereas the control group remained seronegative. None of the pigs in the groups tested induced an HI response against Parvo. The results are indicated here beneath in Table 1.

Table 1 *Post vaccination and post-challenge data for EPL-ID*

Group	Local reactions after boost avg/max diameter	Affected pigs st1/st2	Ph Eur req. for Ery affected pigs	Ery sero response	Parvo HI titre
1	4.9/10 cm	20/30%	< 10%	64	< 4
2	6.7/12 cm	-	-	-	-
3	6.6/9 cm	-	-	-	-
4	0	100%	> 80%	0	< 4

None of the formulations was sufficiently safe (for this test set at 5/7 cm for avg/max local reactions) and the formulation tested for Ery efficacy was also insufficiently efficacious. In addition, this formulation did not induce a Parvo serological response. From the results it can be concluded that EPL is not feasible as vaccine for intradermal vaccination, both due to not meeting the safety and efficacy requirements.

Example 2

Example 2 is a second trial of an Ery-Parvo-Lepto combination vaccine. In this trial, the ery parvo and lepto antigens were the same as in the previous trial. However, instead of formulating all antigens in one unitary vaccine, they were divided over two separate vaccines, namely an EP and an L vaccine. These vaccines were administered simultaneously as separate vaccines into the dermis of pigs using the IDAL device.

For the experiments three different adjuvants were used, the proprietary Emunade® and X-Solve® adjuvants (both oil-in-water adjuvants available from MSD Animal Health, Boxmeer, The Netherlands) and the above identified SVEA adjuvant.

Twenty healthy 18-week-old pigs, not vaccinated against any of the vaccine components, were used. The pigs were divided into 4 groups of 5 animals each. Groups 1, 2 and 3 were vaccinated twice intradermally with a 4-week interval (using the IDAL device) with EP-ID and L-ID (opposite side of the neck), using a vaccine containing 50% antigen content compared to Porcilis EPL (group 3) or 12.5% antigen content compared to Porcilis EPL (groups 1 and 2). The adjuvant Emunade was used in Group1, X-Solve in Group 2 and SVEA in Group 3. Group 4 was vaccinated with Porcilis EPL intramuscularly as a reference vaccination.

After vaccination, all groups were observed for local injection site reactions and serological responses to Ery, Parvo and against 3 of the 5 Lepto antigens i.e. Ictero, Pomona and Tarassovi antigens were determined.

The results are summarized in the Tables 2 and 3 below (responses are in relative units

when no absolute units are given). Note that the parvo titres due to vaccination could not be determined since the animals underwent a field infection, resulting in high titres for all animals.

Table 2 *Post vaccination and post-challenge data for EP-ID*

Group	Local reactions after boost avg/max diameter	Ery sero response	Parvo HI titre
1	2.1/5.0 cm	79	>10 log_2
2	2.6/3.0 cm	112	>10 log_2
3	4.5/7.0 cm	91	>10 log_2
4	0/0 cm	86	>10 log_2

Table 3 *Post vaccination and post-challenge data for L-ID*

Group	Local reactions after boost avg/max diameter	L-ictero	L-pomona	L-tarassovi
1	2.9/6.0 cm	3.8	5.4	2.0
2	1.7/3.0 cm	4.7	6.4	4.4
3	5.3/7.0 cm	nd	nd	nd
4	0/0 cm	6.2	6.7	5.2

For each adjuvant the site reactions were found acceptable and thus the vaccination was deemed safe. However, better results were obtained with the oil-in-water adjuvants, whereas the best results were obtained with the X-solve adjuvant, both regarding safety and efficacy. The latter is a dispersion of oil in water, which unlike Emunade, comprises no aluminium hydroxide.

The Ery antibody responses were comparable for all formulations and at the same level or higher when compared to Porcilis EPL. Parvo vaccination titres typically range between 2 and 10 log_2 whereas after a field infection, titres >10 log_2 may be found. From

the results it is clear that a field infection had occurred during the study and thus no conclusions with regard to vaccination titres are possible. The Lepto antibody responses (Ictero, Pomona and Tarassovi) induced by the different formulations tended to be lower when compared to Porcilis EPL, but still deemed effective (as is known form the use of Porcilis EPL, a positive lepto titer of any level typically equals effective protection).

Example 3

Example 3 is a third trial of an Ery-Parvo-Lepto combination vaccine, aiming at establishing the parvo response of the best vaccine of Example 2, namely the combination vaccine of Group 2, based on the oil-in-water adjuvant X-Solve. Also, the response against PRRS when vaccinating with a live attenuated PRRS vaccine when boosting the EPL prime vaccination was assessed.

Thirty-five healthy 12-week-old pigs weighing not less than 20kg and PRRS negative and seronegative for Ery and Parvo were used. The pigs were divided into 4 groups (10 in each vaccine group and 5 in the control group). Group 1 was vaccinated twice ID (4-week interval) with EP-ID vaccine of Group 2 of Example 2 (right side) in associated non-mixed use with L-ID (of Group 2 of Example 2 (left side). In addition, during the booster vaccination Porcilis PRRS was administered non-mixed intradermally (left side) together with L-ID using Twin IDAL. Group 2 was vaccinated in the same way as group 1, except for the associated use with Porcilis PRRS. Group 3 was vaccinated identical to group 2 except that the EP-ID and L-ID vaccines contained only 25% of the antigen content (thus about 3% when compared with the antigen content of Porcilis EPL). Group 4 was left unvaccinated as negative control. All vaccinations were administered intradermally (0.2ml) in the neck using IDAL or Twin IDAL. After vaccination, groups 1 and 2 were observed for local injection site reactions. At each day of vaccination and at day of challenge blood was collected for Parvo and PRRS serological examination.

At the start of the study, all pigs were seronegative for Ery, Parvo, Lepto and PRRS. The results are indicated here beneath in Table 4 (local reactions indicated corresponding to Tables 2 and 3)

Table 4 *Post vaccination and post-challenge data for EP-ID plus L-ID*

Group	Local reactions EP-ID	Local reactions L-ID	Parvo titre 52 dpv	PRRS titre 52 dpv
1	3.7/6.0 cm	3.8/5.0 cm	6.1	2.0
2	3.6/5.0 cm	3.0/4.0 cm	6.4	0
3	nd	nd	4.0	0
4	0/0 cm	0/0 cm	< 3	0

The site reactions were acceptable. The serology for Parvo and PRRS (established 52 days post vaccination) were acceptable and deemed to meet the efficacy requirements. The PRRS titre post vaccination was comparable with a titre obtainable with the commercial product Porcilis PRRS, and the parvo HI titre was in line with what is obtainable with the commercial product Porcilis EPL.

In conclusion, the associated non mixed use of the separate vaccines EP-ID and L-ID, even when given in conjunction with a live attenuated PRRS vaccine lead to a safe vaccination, effective in protection against and infection with *Erysipelothrix rhusiopathiae*, porcine parvo virus, various Leptospira bacteria and PRRS virus.

Example 4

Example 4 is a fourth trial of an Ery-Parvo-Lepto combination vaccine, aiming at establishing the protection against a L-pomona challenge. Basically, the same experimental set-up as in Example 3 was used, except that in this experiment forty 6-week-old piglets were used, divided over four groups. The vaccination of these groups was identical to the vaccination as described in Example 3.

At 11 weeks of age the pigs were transported to a challenge facility and one week later the pigs were challenged intravenously with *Leptospira interrogans* serogroup Pomona. During 2 weeks after challenge the pigs were observed daily for clinical signs and/or abnormalities in demeanour and appetite. Rectal temperature was measured on regular timepoints until study end which was 2 weeks post-challenge. Serum blood was sampled on day of each vaccination, day of challenge and 2w after challenge and used

to determine antibody titres. Heparin blood was sampled just before challenge, at 24 and 28 hours after challenge and on days 2, 3, 4, 7 and 10 after challenge for re-isolation of challenge strain.

At the start of the study, all pigs were seronegative for Leptospira serovar Pomona and PRRS virus. After the vaccinations the group 1 antibody responses to Ery, Parvo and Lepto were equal to or higher compared to group 2, indicating that there is no negative effect of Porcilis PRRS on the take of EP-ID and L-ID.

Vaccination with EP-ID and L-ID induced small transient local reactions at the vaccine administration site (max diameter 4 and 3 cm, respectively). All reactions had disappeared within 2 weeks after vaccination. The vaccinations did not induce any clinical abnormalities.

The post-challenge results are summarized in Table 5 below.

Table 5 *Post post-challenge data for EP-ID plus L-ID*

Group	Results Lepto challenge: # pos/# total
1 (EP-ID/L-ID/PRRS)	0/10
2 (EP-ID/L-ID)	0/10
3 (EP-ID 25%/L-ID 25%)	0/10
4 (Control)	9/9

The results show that vaccination with EP-ID in associated non-mixed use with L-ID and PRRS, in pigs, is safe. In addition, all three vaccine groups, including associated non-mixed use with the live attenuated PRRS vaccine and the vaccine containing 25% of the normal amount of antigen, induced complete protections against *Leptospira interrogans* serogroup Pomona.

CLAIMS

1. A combination of a first vaccine comprising a non-replicating immunogen of *Erysipelothrix rhusiopathiae* and a non-replicating immunogen of porcine parvo virus, and a second vaccine comprising a non-replicating immunogen of a Leptospira bacterium, for use in prophylactically treating a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium, by associated separate injection of the first vaccine and the second vaccine into the dermis of the pig at a first and a second injection site of that pig respectively.

2. A combination of the first and the second vaccine for use according to claim 1, characterised in that the first and second injection sites are at most 4 cm apart from each other, preferably at most 3 cm apart from each other.

3. A combination of the first and the second vaccine for use according to any of the preceding claims, characterised in that the associated separate injection of the first vaccine and the second vaccine occur simultaneously.

4. A combination of a first and second vaccine for use according to any of the preceding claims, characterised in that the first and second vaccines are given in a prime and boost vaccination scheme, wherein both vaccines are injected at the prime vaccination, and both vaccines are injected at the boost vaccination.

5. A combination of a first and second vaccine for use according to any of the preceding claims, characterised in that at least the first vaccine comprises an adjuvant.

6. A combination of a first and second vaccine for use according to any of the preceding claims, characterised in that both the first and second vaccine comprises an adjuvant.

7. A combination of a first and second vaccine for use according to any of the claims 5 or 6, characterised in that the adjuvant is an oil in water adjuvant.

8. A combination of a first and second vaccine for use according to any of the preceding claims, characterised in that the non-replicating immunogen of *Erysipelothrix rhusiopathiae* is an *Erysipelothrix rhusiopathiae* bacterin, the non-replicating

immunogen of porcine parvo virus is an inactivated porcine parvo virus and the non-replicating immunogen of a Leptospira bacterium is a *Leptospira interrogans* bacterin

9. A combination of a first and second vaccine for use according to claim 8, characterised in that the second vaccine additionally comprises a *Leptospira kirschneri* and/or a *Leptospira santarosai* bacterin.

10. A combination of the first and the second vaccine for use according to any of the preceding claims, characterised in that a third vaccine is injected to the pig, the third vaccine comprising a live attenuated PRRS virus.

11. A combination of the first and the second vaccine for use according to claim 10, characterised in that in the prophylactic treatment, the pig receives a prime vaccination with the first and second vaccine, and a boost vaccination with the first and second vaccine wherein at the boost vaccination the third vaccine is injected as a single dose vaccine.

12. A combination of the first and the second vaccine for use according to claim 11, characterised in that the third vaccine is mixed with the second vaccine.

13. A combination of the first and the second vaccine for use according to any of the preceding claims, characterised in that the first and second vaccine are injected by a jet stream of the respective vaccines using a needle-less device, wherein the jet stream penetrates the skin of the pig.

14. A kit-of-parts comprising a first vaccine comprising a non-replicating immunogen of *Erysipelothrix rhusiopathiae* and a non-replicating immunogen of porcine parvo virus, and a second vaccine comprising a non-replicating immunogen of a Leptospira bacterium, for use in prophylactically treating a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira bacterium, by associated separate injection of the first vaccine and the second vaccine into the dermis of the pig at a first and a second injection site of that pig respectively.

15. A method of prophylactically treating a pig against an infection with *Erysipelothrix rhusiopathiae*, an infection with porcine parvo virus and an infection with Leptospira

bacterium, by associated separate injection of a first vaccine comprising a non-replicating immunogen of *Erysipelothrix rhusiopathiae* and a non-replicating immunogen of porcine parvo virus, and a second vaccine comprising a non-replicating immunogen of a Leptospira bacterium into the dermis of the pig.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/062431

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/02 A61P31/04 A61K39/23
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/099889 A1 (INTERVET INT BV [NL]; INTERVET INC [US]) 7 June 2018 (2018-06-07) page 1, lines 8-14 page 4, line 14 - page 7, line 4 page 8, line 26 - page 9, line 27 ----- -/--	1-15

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
22 July 2024	30/07/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Page, Michael

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/062431

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

International application No
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