MARKETING AUTHORISATION NUMBERING SYSTEM
ADOPTED BY THE EUROPEAN COMMISSION

PORCILIS PESTI

EMEA APPLICATION No	CVMP OPINION No	COMMUNITY REGISTER OF VETERINARY MEDICINAL PRODUCTS No	VETERINARY MEDICINAL PRODUCT PRESENTATION
EMEA/V/C/046/01/0/0	EMEA/CVMP/698/99-Rev. 1	EU/2/99/016/001	Glass bottle containing 50 ml for 25 doses
EMEA/V/C/046/01/0/0	EMEA/CVMP/698/99-Rev. 1	EU/2/99/016/002	Glass bottle containing 100 ml for 50 doses
EMEA/V/C/046/01/0/0	EMEA/CVMP/698/99-Rev. 1	EU/2/99/016/003	Glass bottle containing 250 ml for 125 doses
EMEA/V/C/046/01/0/0	EMEA/CVMP/698/99-Rev. 1	EU/2/99/016/004	Polyethylene terephthalate bottle containing 50 ml for 25 doses
EMEA/V/C/046/01/0/0	EMEA/CVMP/698/99-Rev. 1	EU/2/99/016/005	Polyethylene terephthalate bottle containing 100 ml for 50 doses
EMEA/V/C/046/01/0/0	EMEA/CVMP/698/99-Rev. 1	EU/2/99/016/006	Polyethylene terephthalate bottle containing 250 ml for 125 doses

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PRODUCT PROFILE

Product name:	Porcilis Pesti
Procedure No.:	EMEA/V/C/046/01/0/0
Applicant company :	Intervet International bv Wim de Körverstraat 35 P.O. Box 31 5831 AN Boxmeer The Netherlands
Active substances	Classical Swine Fever Virus (CSFV) - E_2 subunit antigen
Pharmaceutical form:	Emulsion for injection
Strength	120 Elisa Units (EU) per dose of 2 ml
Presentation:	Bottles of type I hydrolytic glass or polyethylene terephthalate (PET) containing 50 ml for 25 doses, 100 ml for 50 doses and 250 ml for 125 doses.
Target species:	Pigs
Withdrawal period:	Zero days
Route of administration:	Intramuscular injection
Product type:	Immunological
Therapeutic indication:	Active immunisation of pigs from the age of 5 weeks onwards to prevent mortality and to reduce clinical signs of Classical Swine Fever, as well as to reduce infection with, and excretion of, CSF field virus.

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SCIENTIFIC DISCUSSION

1. INTRODUCTION

Porcilis Pesti is a water-in-oil emulsion containing as the active component 120 ELISA Units (EU) of the classical swine fever virus (CSFV) E2 antigen (subunit vaccine) per dose of 2 ml.

The indication for Porcilis Pesti is active immunisation of pigs from the age of 5 weeks onwards to prevent mortality and to reduce clinical signs of Classical Swine Fever, as well as to reduce infection with, and excretion of, CSF field virus.

By using only the immunodominant E2 antigen of CSFV in the vaccine, vaccinated animals will only develop an immune response against E2, including antibodies to E2. In contrast animals infected with field virus will develop antibodies to all immunodominant antigens of CSFV e.g. E2, E^{RNS} and NS3. By using proper diagnostic tools, this marker property of Porcilis Pesti makes it possible to distinguish from each other the following groups of pigs: non vaccinated-non infected (negative in both the anti-E2 and the anti E^{RNS} antibody test), vaccinated non-infected (positive in the anti-E2 and negative in the anti E^{RNS} antibody test), and infected (positive in both tests, whether vaccinated or not vaccinated). After infection it takes a few days before antibodies against these antigens can be detected. In addition antibodies against both antigens may be detected in animals which have recovered from acute infection.

The volume of a single dose is 2 ml, to be injected by the intramuscular route. The primary vaccination schedule consists of 2 doses, the second dose being given after a 4-week interval. Revaccination with a single dose is recommended at 6-month intervals.

The CSFV-E2 antigen is produced in an insect cell baculovirus expression system. The baculovirus has been genetically modified to introduce the CSFV gene encoding the CSFV-E2 antigen. The E2 antigen is secreted in the antigen production medium. Porcilis Pesti was eligible for the granting of a Community marketing authorisation via the centralised system since it is constructed via biotechnological techniques and therefore is in accordance with Part A of the Annex of Council Regulation (EEC) No 2309/93.

Two preliminary meetings have been held at the EMEA, the first one with representatives of the company, the second one with representation of DGVI of the European Commission (this representation now falls under DG SANCO).

There are three main conclusions resulting from the discussions during these meetings:

1. For obvious epidemiological reasons the company is unable to produce results on the efficacy of the product derived from field trials; only safety field trials were authorised by the competent authorities of the European Union in accordance with disease control and/or vaccination policy for Swine Fever.

2. For Porcilis Pesti, being a subunit vaccine, at least two antigens may be chosen as markers. Therefore several diagnostic companies apart from the vaccine manufacturer may introduce to the market different diagnostic tests; consequently, the company has only to provide scientific evidence that the product works effectively as a marker vaccine.

3. The use of such a vaccine must be sanctioned by DG SANCO competent authorities. It was therefore decided that the CVMP would give an opinion on the quality, safety and efficacy characteristics of the product, and that DG SANCO would be provided with the

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assessment report on day 120 of the assessment period including the CVMP list of questions to the Applicant. DG SANCO would therefore need to decide if, for epidemiological purposes, to confirm satisfactory clinical efficacy under field conditions, further data is required, from the company, on the basis of the CVMP assessment report.

2. OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

The main quality issues specific for this vaccine are:

- the novel baculovirus/insect cell production system
- the stability of the antigen in the water-in-oil adjuvant suspension

A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

	Names of ingredients	Quantity per 2 ml (= 1 dose)	Function	Reference
Active ingredient	CSFV-E2 antigen	120 EU*	Induction of immunity	See section II.C.

Adjuvant	Light liquid paraffin	941.4 mg	Adjuvant	Ph.Eur. 0240
Excipients	Polysorbate 80		Emulsifier	Ph.Eur. 0428
	Sorbitan oleate		Emulsifier	Ph.Eur. 1041

* contained in a maximum of 810.8 mg antigen production medium. The antigen may have been brought on strength by adding water for injection. A trace of gentamicin may be present as a remnant from antigen production.

Presentation
Each bottle of final product contains 50, 100 or 250 ml, corresponding to 25, 50 or 125 doses of 2 ml.

Container:

The containers are of glass, Ph.Eur. type I, or polyethylene terephthalate (PET).

The PET-containers are produced in accordance with the requirements of the American Food and Drug Administration (FDA) and the German Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV) (21CFR 177.1315, April 1, 1991 revision, and the EEC-directive 90/128/EEC of February 1990).

The containers are closed with a nitryl rubber stopper, Ph.Eur., and sealed with a coded aluminium cap.

Development Pharmaceutics

Choice of the vaccine antigen

The E2 structural protein was chosen as the vaccinating antigen because it is an immunodominant antigen and antibodies to E2 are strongly virus neutralizing and were shown to be protective, also in the absence of antibodies to other structural proteins of CSFV (E^{RNS}).

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To test the effect of the addition of E^{RNS} antigen, comparative vaccination/challenge experiments were performed in which the degree of protection provided by an E2 based vaccine was compared with E2 + E^{RNS} based vaccines. Although, based on serology (Virus Neutralisation - VN - titres), the combined vaccine performed better than the E2 antigen only, this difference was not reflected in the level of clinical protection afforded by both vaccines which was 100% in both cases as judged by clinical signs, mortality, viraemia and temperature. In order to achieve a similar VN antibody response with monovalent E2 vaccine to that observed with the combination vaccine, the antigen content of the vaccines was increased to the level applied for in the dossier.

Furthermore, the use of monovalent E2 antigen allows the development of an appropriate test to use the vaccine as a marker vaccine.

Adjuvant selection

Experiments were set up in which alternative adjuvant concepts, suitable for large scale production of a commercial vaccine, were tested.

Both with respect to the onset of the serological response and with respect to titre, oil emulsion adjuvants were found to be the most suitable.

Antigen dose, vaccination schedule

Based on the serological data in three experiments, it was found that the increase of the antigen content from 30-40 ELISA Units (EU) to a content of approximately 86-100 EU CSF-E2 antigen per dose resulted generally in higher group mean titres and, even more importantly, in almost 100% of individual animals reacting with a VN titre above 6 $\log_2$. This led to the conclusion that Porcilis Pesti should optimally contain an antigenic dose, which would be in the order of 100 EU CSF-E2 subunit antigen per dose of 2 ml.

All studies discussed above used the basic vaccination schedule, which consisted of two vaccinations at a 4-week interval.

To develop a vaccine and vaccination scheme which leads to a clinical protection as complete as possible as well as to absence of viraemia and virus excretion, the vaccine was eventually standardised on an antigen content of 120 EU CSF-E2 subunit antigen per dose and to recommend two vaccinations at a 4-week interval as the basic vaccination.

Preservative

Although Porcilis Pesti is a multiple dose presentation, no preservative has been added for the following reasons:

1) To exclude potentially toxic excipients from Immunological Veterinary Medicinal Products (IVMPs) where possible, as described in 'Inclusion of antimicrobial preservatives in immunological veterinary medicinal products' published in Volume 7B of the rules governing medicinal products in the European Union.
2) Production is executed under GMP (it is a sterile product).
3) The delivery method is a closed system.
4) The relatively short time (≤ 2 hour) needed to administer the contents of the whole bottle minimizes the chance of biological contamination.

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Choice of the baculovirus expression system and of the insect cell line for baculovirus propagation

As stated the main reason for choosing this system is its high antigen productivity, which is essential in the commercial production of an effective subunit CSF vaccine. Other important aspects are that by using an eukaryotic expression system, post-translation modification (glycosylation) takes place, which is mimicking the native circumstances of E2 processing during replication of the virus. Within the eukaryotic expression systems, the advantage of the baculovirus expression system is that serum free medium can be used in the antigen production step which is relevant both with respect to safety of the vaccine and cost reduction since no further downstream processing is required.

B DESCRIPTION OF METHOD OF PREPARATION

The production procedure is described as two phases: firstly the production of the E2 subunit antigen in the baculo expression system, and secondly the formulation of the final product.

II.B.1. Method of preparation

The production of the E2 subunit antigen

Production of the E2 subunit antigen is based on fermentation of *Spodoptera frugiperda* cells infected with the recombinant baculovirus vector. The antigen is secreted into and harvested from the production medium, processed and treated with ß-propiolactone (BPL) for virus-inactivation.

The fermenter sizes used for clean cell production range between 500 litres and 1000 litres. The production scale has a two-fold margin. The fermenters are equipped with systems for automatic measurement, control and registration of: pH, pO_2, temperature and stirring rate.

The CVMP was concerned that there was a 10-fold variation of the production scale without proper validation. In reply, the Applicant indicated that the lower scale limit will be raised from 100 to 500 litres. The production scale is herewith narrowed to only a 2-fold margin.

Liability to disruption

The antigen is not liable to disruption since for removal of cell debris only, the antigen harvested is subjected to a low speed centrifugation step (800 g).

Production of the final vaccine

Antigen batches are selected based on the antigenic mass value as determined by ELISA. If necessary, water for injection is added to obtain the required concentration of 120 EU/dose. The mixture of the selected antigen batches constitutes the antigen fraction.

The oil soluble constituents are dissolved in the liquid paraffin and the solution is filtered sterile. The antigen fraction is added aseptically to the aqueous solution and stirred until homogeneous suspension is obtained.

The uniform dispersal of the antigen containing water phase in the oil fraction has been validated on production scale; the observations being documented by photographs (microscopic). The relevant parameter is that the water phase containing the E2 antigen is evenly distributed over the field of vision (400 x magnification) and that the droplet size

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generally is less than 5 μm when the vaccine is diluted 4 times in liquid paraffin (1000 x magnification).

Purification

The antigen is semi purified by a centrifugation step and membrane filtration in which cell debris is removed.

In comparison to the antigens used in most other veterinary vaccines, the E2 antigen is relatively pure because of the production system applied:

- The culture medium used for E2 production is completely protein free.
- There is only one expression product: E2.

All production procedures are performed according to standardised methods.

For these reasons no further purification of the antigen is performed.

The CVMP nevertheless requested the impurity profile of the antigen harvest determined on 5 representative antigen production batches (fermentation scale of 500 l to 750 l). This was provided and the data enable characterisation of the E2 antigen production. The results show a consistent profile and confirm production consistency.

Filtration

In addition to integrity testing of the sterilising filter before and after use by both the foreward flow and bubble point methods, the filter is validated using the *Pseudomonas (=Brevundimona) diminuta* method.

Validation of the production method

Validation of the BPL treatment

To guarantee complete inactivation of the baculovirus, the same requirements are applied as for conventional whole virus inactivated antigens. This means that the inactivation has to be completed within 67% of the total period of inactivation. The report presenting the results of the kinetics of baculovirus inactivation showed that they comply with the requirements as laid down for whole virus inactivated vaccines.

Following harvest, the supernatant is centrifuged and then treated with Beta-propiolactone (BPL) to kill off any potential baculovirus contaminants. The antigen in virus-free supernatant is stored for at least 24 hours at 2-8°C before being incorporated in the emulsion system. The antigen is not oxygen labile. It has been shown that BPL-treated antigen which had been stored for approximately 16 months at 2-8 °C before it was used in vaccine formulation resulted in a vaccine with adequate potency (100% $\geq$ 6.0 $\log_2$ VN). This shows that the antigen is stable under the conditions of storage for 16 months, which is the proposed maximum storage period for the BPL-treated antigen.

A justification was provided why the inactivation procedure was not validated using a range of model viruses. The inactivation procedure has been validated for the recombinant baculovirus. Production is furthermore performed under GMP conditions. There are no indications that any of the starting materials used in the production of the E2 antigen contains an adventitious virus. So the only organism present in the harvest is the baculovirus. The CVMP therefore accepted that there is no further need to validate the inactivation of other

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viruses than the production virus.

Freedom from extraneous agents of the virus seed lot, the cell seed lot and the foetal calf serum is considered below:

- **Virus seed lot and Cell seed lot**
  Both the virus seed lot and the cell seed lot have been thoroughly tested and shown to be free of all agents listed in guideline III/3427/93 – species porcine.
- **Foetal Calf serum**
  Before use of the foetal calf serum, it is treated by γ-irradiation applying an absorbed dose between 25 and 50 kGy. This inactivation method has been validated.

Validation of the antigen production method

In-process test data of consecutive batches are presented below.

Antigen batch	Test **IPC-01** (Antigenic mass)	Test **IPC-02** (Sterility)	Test **IPC-03** (Inactivation)
CSF 97.20.015 Y	≥ 100 EU/ml	No growth detected	No infectious virus detected
CSF 97.20.015 Z	≥ 100 EU/ml	No growth detected	No infectious virus detected
CSF 97.20.015 AA	≥ 100 EU/ml	No growth detected	No infectious virus detected
CSF 97.20.015 AB	≥ 100 EU/ml	No growth detected	No infectious virus detected
Specification	≥ 50 EU/ml		

The consistency of the fermentation parameters was demonstrated through monitoring of fermentation during 5 days for 3 batches of antigen, which were produced at a scale of 500 and 750 litres.

The specification for antigenic mass was increased to > 100 EU/ml since the CVMP considered the initial 50 EU/ml too low in view of the batch analysis results.

Validation of the method to produce final product

The quality control results of four consecutive batches are presented below. Antigen from four different fermentation batches was used, however with some overlap. The consistency of potency for different fermentation batches was furthermore demonstrated in providing results for a fifth batch.

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Batch	Results obtained in Quality Control							
	Pharmaceutical tests				Sterility	Inactivation	Safety (after double dose injection) and marker property (after repeated dose injection)	Potency
	Appearance	Type of emulsion	Pharmaceutical stability	Viscosity				
77339 (162 EU)	White to nearly white emulsion	water-in-oil	emulsion stable for 3 weeks at 37°C	118 cP	Sterile	No residual infectivity detected	No abnormal local or systemic reactions observed. Sera negative for CSFV E^{RNS} antibodies by specific ELISA	80%
77340 (162 EU)	White to nearly white emulsion	water-in-oil	emulsion stable for 3 weeks at 37°C	115 cP	Sterile	No residual infectivity detected	No abnormal local or systemic reactions observed Sera negative for CSFV E^{RNS} antibodies by specific ELISA	80%
77341 (122 EU)	White to nearly white emulsion	water-in-oil	emulsion stable for 3 weeks at 37°C	85 cP	Sterile	No residual infectivity detected	No abnormal local or systemic reactions observed. Sera negative for CSFV E^{RNS} antibodies by specific ELISA	100%
77342 (122 EU)	White to nearly white emulsion	water-in-oil	emulsion stable for 3 weeks at 37°C	88 cP	Sterile	No residual infectivity detected	No abnormal local or systemic reactions observed. Sera negative for CSFV E^{RNS} antibodies by specific ELISA	100%
Release require-ments	White to nearly white emulsion	water-in-oil	emulsion stable for 3 weeks at 37°C	≤ 160 cP	Sterile	No residual infectivity detected	No abnormal local or systemic reactions may be observed. Sera negative for CSFV E^{RNS} antibodies by specific ELISA	≥ 80%

The PET containers are γ-irradiated applying an absorbed dose of at least 10 kGy. The glass containers by dry heat sterilisation for at least 1 minute at 250°C.

The viscosity was reviewed. Since on average the vaccine has a viscosity of 102 cP with a standard deviation of 19 the specification for viscosity was set at ≤ 160 (mean + 3 x SD).

C CONTROL OF STARTING MATERIALS

Starting materials listed in a Pharmacopoeia

The starting materials listed in a Pharmacopoeia comply with current versions of the monographs, with the exception of a light liquid paraffin component.

The liquid paraffin used has a viscosity of 11-14 cP. This results in a final product with a viscosity, which is in the range of 85 to 137 cP. When liquid paraffin with a viscosity of 25-80 cP, as specified in the Ph. Eur. monograph would be used the final product would have a

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considerably higher viscosity. Experimental data show that the use of liquid parrafin with a viscosity of 25-80 cP would result in Porcilis Pesti vaccine with a viscosity of approximately 1100 cP. Such a vaccine has an unacceptable syringeabiliy, and most probably also results in more severe local reactions. For these reasons liquid paraffin is used which has a lower viscosity than specified in the Ph. Eur. but with regards to all other parameters fully complies with the Ph. Eur. and this is considered acceptable.

Starting materials not listed in a Pharmacopoeia

a) Biological origin

The following starting materials are of biological origin and deserve special attention:

1) Sf-21-CB insect cell line
2) recombinant baculovirus
3) Foetal calf serum

1) Sf-21-CB insect cell line and cell bank system

Sf-21-CB is a *Spodoptera frugiperda* insect cell line, which is used as the substrate to propagate the recombinant *Autographa california* Multiple Nuclear Polyhedrosis Virus expressing the CSFV-E2 antigen (AcMNPV-CSFV-E2 p10 clone 2A). After 28 passages the cells (now coded Sf-21-CB) were collected and frozen down as the Master Cell Stock (MCS) stored in liquid nitrogen. 650 x 1 ml aliquots of the MCS were prepared.

Testing of the MCS is performed according to the European Pharmacopoeia General text 5.2.4 "*Cell cultures for the production of veterinary vaccines,* CELL LINES*"* and the Note for Guidance on extraneous agents (III/3427/93):

• Identification of species	• AuthentiKit
• Karyology	• photographs of chromosome spreads of the low and high passage levels (MCS+5 and MCS+19)
• Bacteria and fungi	• European Pharmacopoeia 2.6.1
• *Brucella suis*	• Specific test
• Mycoplasmas (non avian)	• European Pharmacopoeia 2.6.7
• General microscopy	

- Extraneous agents as summarised in the table below:

Result of Tests	Interpretation
No cytopathic effect or haemadsorption observed in PK, SK and BHK cell cultures	<u>absence of</u>: Aujeszky's disease virus (*no CPE on PK, SK*) encephalomyocarditis virus (*no CPE on PK, BHK*) haemagglutinating encephalomyelitis virus (*no CPE+HA on PK*) transmissible gastroenteritis virus (*no CPE on PK*) porcine respiratory coronavirus (*no CPE on PK*) porcine adenovirus (*no CPE on PK, SK*) porcine enterovirus (incl. teschen-talfan) (*no CPE on PK, SK*) porcine vesicular exanthema virus (*no CPE on PK*) swine poxvirus (*no CPE on PK*) swine vesicular disease virus (*no CPE on PK, SK*)
No virus detected in specific tests	<u>absence of</u>: bovine viral diarrhoea virus (*no IF on BEL and BT*)

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	classical swine fever virus (*no IF on SK*) foot and mouth disease virus (A, O, C, ASIA1, SAT1, SAT2, SAT3) (*ELISA negative on BHK*) porcine influenza virus (*no HA on allantoic fluid*) porcine parvovirus (*no IF on SK,*) porcine cytomegalovirus (*no CPE and IF on PLM*) porcine respiratory and reproductive syndrome virus (*no CPE and peroxidase staining on PLM*) rabies virus (*no CPE on BHK*) vesicular stomatitis virus (*ELISA negative on BHK*)

Note: A specific test for African swine fever virus was considered not necessary because this agent is not present in the country of origin of the cell seed.

Each new working virus seed lot will be tested for absence of adventitious viral contamination by inoculating a sample of the seed on porcine kidney cells. The general tests applied will be examination for CPE and a test for haemagglutinins.

Sequence data have been collected from the MSV and from MSV 5 produced on MCS+20. The data show that the construct is genetically stable when using the maximum passage levels of both the virus and the cells.

Characterisation of the E2 antigen produced by the recombinant baculovirus is included in the dossier. A report was provided including Western blots prepared from the PAGE gels in which the E2 band is visualised by using both a monoclonal antibody directed against CSFV-E2 and a polyclonal monospecific rabbit serum directed against CSFV-E2 antigen.

Consistency of production is guaranteed by the seed lot systems. The genetic stability of the construct has been demonstrated. The identity of the E2 antigen is confirmed specifically and unambiguously by the antigenic mass ELISA which is performed on each batch of antigen. However, of most relevance is the potency test, performed on each batch of vaccine. This test shows whether the vaccine can elicit the E2 antibody titres as measured in the VN assay which have shown to correlate with protection.

In order to establish the production consistency in terms of purity profile, data from five further batches of E2 antigen at production scale were provided. The purity profile was found identical in these five batches.

The CVMP was concerned that testing for infectious retroviruses was only performed by using electron microscopy (C- and D-type particles), which is considered having a low sensitivity as a test for the detection of retroviruses. The usual method to test for absence of retroviruses is to test the substance for absence of RT activity. The Applicant explained that for insect cells and also for insect viruses which have been grown on these insect cells, the RT test is not suitable to test for absence of retroviruses because of an endogenous RT background activity. Furthermore, to date, no members of the family Retroviridae have been detected in insects and testing for porcine retroviruses is not required by the note for guidance on extraneous agents (III/3427/93).

The MCS was cultivated for 28 days and examined for absence of extraneous pathogens by checking the cultures for CPE and absence of haemadsorption. Different neutralisation attempts of the MSV were without success because of the characteristics of the baculo virus.

The possibility of contamination with arthropod viruses can be disregarded since especially for Lepidopteran cells the arthropod viruses are incapable of replicating in vertebrate cells. Therefore, even if the harvest was not treated with 0.5% BPL this would not pose any risk to

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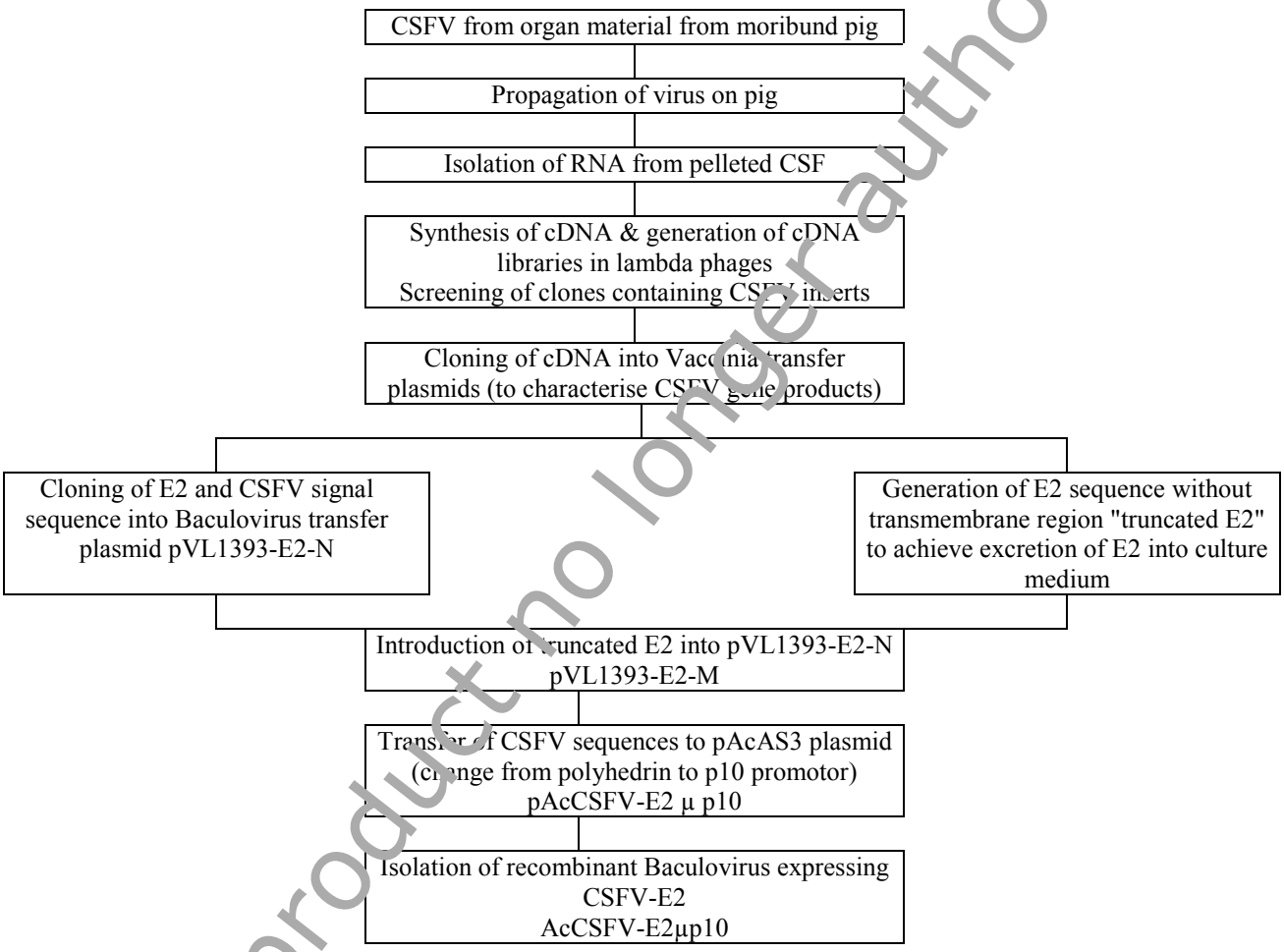
the target animal. An exception to this could be vesicular stomatitis virus which has a very wide host range and which can grow on Lepidopteran cells. However, this virus has been tested in both the virus seed lot and the cell seed lot by specific tests with negative results.

2) Recombinant *Autographa california* Multiple Nuclear Polyhedrosis Virus expressing the CSFV-E2 antigen (AcMNPV-CSFV-E2 p10 clone 2A)

The construction of this baculovirus vector is summarised below.



The virus was passaged once in Sf cells. The supernatant from this cell culture passage (= MSV level) was harvested and aseptically aliquoted in 964 x 1 ml in ampoules and flame sealed. This is the MSV for the production of the active ingredient in Porcilis Pesti. It is coded with: *Clone 2A, AcCSFV-E2 p10, Master Seed Virus, CSF9720015M* and stored at -70°C.

This Master Seed Lot has been tested according to the European Pharmacopoeia Monograph 0062 "*Vaccines for veterinary use*", and the Note for Guidance on extraneous agents (III/3427/93):

• Bacteria and fungi	• European Pharmacopoeia 2.6.1
• *Brucella suis*	• Specific test
• Mycoplasmas (non avian)	• European Pharmacopoeia 2.6.7

- Extraneous agents as summarised in the table below:

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Result of Tests	Interpretation
No cytopathic effect or haemadsorption observed in PK, SK, BEL and BHK cell cultures	<u>absence of</u>: Aujeszky's disease virus (*no CPE on PK, SK*) encephalomyocarditis virus (*no CPE on PK, BHK*) haemagglutinating encephalomyelitis virus (*no CPE+HA on PK*) transmissible gastroenteritis virus (*no CPE on PK*) porcine respiratory coronavirus (*no CPE on PK*) porcine adenovirus (*no CPE on PK, SK*) porcine enterovirus (incl. teschen-talfan) (*no CPE on PK, SK*) porcine vesicular exanthema virus (*no CPE on PK*) swine poxvirus (*no CPE on PK*) swine vesicular disease virus (*no CPE on PK, SK*)
No virus detected in specific tests	<u>absence of</u>: bovine viral diarrhoea virus (*no IF on PEL*) classical swine fever virus (*no IF on SK*) foot and mouth disease virus (A, O, C, ASIA1, SAT1, SAT2, SAT3) (*ELISA negative on BHK*) porcine influenza virus (*no HA on allantoic fluid*) porcine parvovirus (*no IF on SK*) porcine cytomegalovirus (*no CPE and IF on PLM*) porcine respiratory and reproductive syndrome virus (*PCR*) rabies virus (*no IF on BHK*) vesicular stomatitis virus (*ELISA negative on BHK)*

A specific test for African swine fever virus was considered not necessary because this agent is not present in the country of origin of the virus seed. The country of origin is Germany.

A working seed lot can be prepared at any level between MSV+1 and MSV+4 by propagation of the preceding virus level in Sf cell culture. The virus containing supernatant would be harvested and filled in suitable aliquots in ampoules, vials or bottles and may be freeze-dried. Depending on the passage level working seed lots would be stored at -70°C (wet seeds) or at -25°C (freeze-dried seeds). Each seed lot would be identified with an unique code and tested as follows:

• General microscopy	
• Bacteria and fungi	• European Pharmacopoeia 2.6.1
• Mycoplasmas	• European Pharmacopoeia 2.6.7

3) Foetal calf serum

On the grounds of possible TSE contamination the country of origin is restricted to New Zealand, Australia, Canada or United States of America.

After release for sales by the supplier, but before entry in the company's stocks, batches of serum are subjected to γ-irradiation at a temperature below -40°C [in solid carbon dioxide] applying an absorbed dose between 25 and 50 kGy as an extra safety measure.

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The specified viruses the supplier is searching for are Infectious Bovine Rhinotracheitis Virus, Bovine Parainflueza –3 virus and Bovine Viral Diarrhoea Virus. If the serum is sourced from areas where blue tongue is endemic, (e.g. Australia) also a test for blue tongue virus is performed.

Gamma irradiation has been validated by the Applicant using the most notorious contaminant of bovine serum i.e. Bovine Viral Diarrhoea Virus (BVDV). Other parties have validated the procedure for a panel of 5 bovine viruses, that encompasses all major bovine serum contaminants.

On entry the appearance and the presence of proof of γ-irradiation and of the suppliers certificate are checked.

Assurance that the donor animals comply with note for guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products (EMEA/CVMP/145/97-REVISION) was provided.

Tests

by the supplier

- Bacteria and fungi: sterile
- Mycoplasma: none found
- Adventitious viruses: specified viruses not found

The supplier provides a certificate showing the country of origin, the tests performed and their results.

by the Applicant

- Cell growth promoting quality is tested
- Freedom of inhibitors of growth of specific viruses is tested if required for the production system

b) Non-biological origin

ß-propiolactone (BPL) is a highly effective virucidal agent which is completely safe after hydrolysis.

A copy of the Certificate of Analysis was provided. On receipt the identity is confirmed by HPLC. The retention time which should be similar as those obtained for a standard. BPL is not subject to a pharmacopoeial monograph. The test performed by the supplier is on content of BPL, which should be above 95 %.

C Media

The complete qualitative and quantitative composition of culture media used for production are presented.

C 1. Medium used to produce the production cells

The required amount of medium is supplemented with:

- Gentamicin sulphate (Ph. Eur.)
- Natamycin (USP)
- Glucose (Ph. Eur.)
- Foetal calf serum

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C 2. Medium used to produce the CSFV-E2 antigen

The required amount of medium is supplemented with:

- Gentamicin sulphate (Ph. Eur)
- Glucose (Ph. Eur.)

Certificates of analysis for the glucose and gentamicin sulphate which are used as medium constituents for antigen production were provided.
The cell production medium and the virus production medium are sterilised by 0.22 µm filtration.

D CONTROL TESTS CARRIED OUT AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

D.1. Production and in-process control of the E2 subunit antigen

Antigen mass, sterility and completeness of the baculovirus inactivation are checked.

Control cells will be run in parallel to the production cells. At the end of the fermentation process, these cells will be examined for CPE. No CPE should be observed.

D.2. Production and in-process control of the final vaccine

Confirmation of the completeness of the baculovirus inactivation, check on correct fill volume, check on physical aspects, sterility test, check on safety and marker property and potency test are performed.
It was considered that adequate in-process controls are in place for the production of the final product.

E CONTROL TESTS ON THE FINISHED PRODUCT

Test	Specification
Visual appearance	The homogeneous emulsion should be nearly white.
Type of emulsion	A sample of the product should be shown to be a water-in-oil emulsion.
Pharmaceutical stability	Upon storage of a sample of the product for 3 weeks at 37°C, the emulsion should not break.
Viscosity	The viscosity as measured at 25°C with a Brookfield rotation viscosity meter should be below 160 cP.
Sterility test	The vaccine must comply with the Ph.Eur.
Safety and marker property The test is performed in accordance with the Ph.Eur. monograph 0062.	After the double dose injection no abnormal local or systemic reactions may be observed. The vaccine should not elicit antibodies against CSFV-E^{RNS} antigen.

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Potency Five piglets are vaccinated twice with an interval of 4 weeks. Blood samples are taken at 2 weeks after last vaccination for the determination of the VN titre. The titre of neutralizing antibodies in the serum is defined as the reciprocal of the highest dilution of the specimen showing absence of virus replication.	The vaccine should induce an antibody titre of ≥ 6 $\log_2$ VN units in at least 80% of the pigs.

The Applicant has agreed to tighten the specification for the potency test after the CVMP required more stringent potency specifications, based on a larger database.

New specification:

a) 80% of the piglets have a VN titre of ≥ 6 $\log_2$
and
b) 100% of the piglets have a VN titre of ≥ 5 $\log_2$

This more stringent extra criterion will further enhance the quality of the batches.

Quality of a batch containing 43 EU/dose and differentiating capacity of the batch test with the original specification.

The original batch test was able to differentiate between effective ($\geq$ 43 EU per dose) and ineffective ($\leq$ 15 EU per dose) batches.

Rationale for the new specification

As shown in the duration of immunity experiment, a low VN titre does not correlate with no protection per se. Pigs with titres as low as 2.5 $\log_2$ VN, as measured 6 months after the vaccination, were found well protected against a severe challenge. However, it is highly probable that also in these pigs the titres were higher shortly after the vaccination and that this is indeed a relevant aspect in protection. In one experiment, a pig vaccinated with vaccine containing an antigenic content as low as 5 EU/dose was found not to be protected in spite of having a VN titre of 4.0 $\log_2$ VN. This seems to be on the threshold since another pig in the same group was found protected at a titre of 3.5 $\log_2$ VN. The Applicant therefore has decided to tighten the potency test specification in that 80% of the pigs must have a VN titre of $\geq$ 6.0 $\log_2$ VN and that 100% of the pigs must have a titre of $\geq$ 5.0 $\log_2$ VN.

F STABILITY

Stability of the bulk antigen

Data on three lots of untreated bulk antigen after storage at -80°C for one week and at 4°C for up to 16 months have been submitted. No significant loss in antigen content as measured by ELISA was detected.

Additional data provided show that the final vaccine prepared from E2 antigen which has been stored for 16 months at 2°C –8°C had a potency which passed the new requirement of 80% $\geq$ 6.0 log2 VN and 100% $\geq$ 5.0 log2 VN. This result confirms that the E2 antigen can be stored for a maximum period of 16 months at 2°C –8°C.

Stability tests on the finished product

Stability testing data of different batches were provided at the time of the original dossier and

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in the answers to the list of questions. Fifteen months data will become available by June 2000.

At present, satisfactory potency data have been obtained with 2 batches after storage for 17 months in glass type I and 20 months in the PET container. Both these batches were produced at production scale (900 kg and 840 kg). Based on these data and the commitment to collect data on a third batch produced to contain the standard antigen content of 120 EU per dose, a shelf life of 12 months for vaccine filled in glass type I or filled in the PET container is proposed.

The Applicant provided a letter of commitment to submit stability data before the end of June 2000 for a third batch held in PET containers at a standard antigen content of 120 EU.

The PET container is tested according to USP 23; <661> 1782-1786. Certificates showing compliance with the USP were provided. Further documentation provided shows that the PET containers are in conformity with the general requirements of Ph.Eur monograph 3.2.2.

The product has not been tested for light sensitivity. There are no reasons to expect a negative effect since the product does not contain live micro-organisms. The vaccine has to be stored in a refrigerator at 2°C –8°C, which is in the dark.

In-use shelf-life

In one experiment, the in use shelf life of 3 hours has been validated by performing a potency test in pigs (2 vaccinations with an interval of 4 weeks; serology 2 weeks after the second vaccination). The test was combined with an accelerated stability test as follows:

Before the first vaccination, product (batch 73452) was stored in a closed container for 3 days at 30°C. After this period the container was broached and left at room temperature for 3 to 3.5 hours. Using a new container, this procedure was repeated for the second vaccination. Using vaccine from these two containers, the potency was tested in parallel with the same vaccine, for which both containers had been stored at 2-8°C throughout. The results, presented in the table below, show that the vaccine retains its potency when stored closed for 3 days at 30°C followed by 3 hours at room temperature (two sample *t*-test on the VN titres: p = 0.40). Transport at 2-8°C is not necessary therefore and the vaccine retains its potency for at least 3 hours after the container has been broached.

VN titre (log2) in pig:	Storage conditions	
	3 days closed at 30°C, broached then 3-3.5 hours at room temperature	at 2-8°C throughout (=reference)
1	6.5	9.0
2	8.5	7.0
3	6.5	6.5
4	6.5	7.5
5	8.0	9.0
Potency	100% > 5 100% > 6	100% > 5 100% > 6

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3. OVERVIEW OF PART III OF THE DOSSIER: SAFETY TESTING

Introduction

Relevance of the data

Six studies were provided which document the safety under laboratory conditions (4 studies) and under field conditions in finishing pigs (1 study) and in lactating and pregnant sows (1 study).

The safety studies were performed in parallel with the efficacy studies performed to establish the standard antigen dose of 120 EU per dose of 2 ml. Therefore, data are available with vaccine formulated to contain 43, 120 and 210 EU per dose. In all cases the pharmaceutical formulation of Porcilis Pesti was used.

Completeness of safety data for each of the classes and species for which the product is intended

The only target animal is the pig. All experiments involved pigs treated with Porcilis Pesti by the recommended intramuscular route. The laboratory experiments and one field trial were performed with piglets at an age of 6 to 12 weeks, whereas another field trial was conducted with sows irrespective of their reproductive cycle.

LABORATORY TESTS

Four laboratory safety studies have been performed. Two of these were specific safety studies in which the safety of the administration of a single dose, a double dose and repeated administration was investigated.

The other two laboratory experiments were combined safety and efficacy studies, which provide safety data after the administration of one dose and a repeated dose.

1. **Safety of the administration of one dose**
2. **Safety of one administration of an overdose**

The two specific laboratory safety experiments with Porcilis Pesti containing 210 EU CSFV-E2 antigen and Porcilis Pesti containing 43 EU CSFV-E2 antigen respectively, were performed with a double dose (4 ml) followed 2 weeks later by a single dose (2 ml).

In the experiment for testing the safety of one dose, an overdose and the repeated administration of a dose was investigated with Porcilis Pesti batch 77335 containing 210 EU CSFV-E2 subunit antigen per dose of 2 ml. A group of three, 7 to 8 week old piglets was vaccinated intramuscularly with an overdose (4 ml) followed by one dose (2 ml) two weeks later. No systemic reactions were observed. Body temperature stayed within normal limits (group mean between 39.0 - 40.0 °C). A minor reaction was palpable at the injection site in 2 pigs for a maximum of 2 days after the first vaccination. No local reactions were observed after the second vaccination. Macroscopical examination of the injection site at two weeks post vaccination revealed no abnormalities.

In the second experiment safety of one dose, an overdose and the repeated administration of a dose was investigated with Porcilis Pesti batch 27367 containing 43 EU CSFV-E2 subunit antigen per dose of 2 ml. A group of ten 6-week-old piglets was vaccinated intramuscularly with an overdose (4 ml) followed by one dose (2 ml) two weeks later. Five similar pigs were kept as unvaccinated controls. No systemic reactions were observed. Body temperature stayed within normal limits. Body weight gain from first vaccination until 14 days post second

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vaccination was better in vaccinates than in controls (group means: vaccinates weight gain 577 g/day, controls 539 g/day). A minor reaction was palpable at the injection site in 4 pigs only at 4 hours after the first vaccination. After the second vaccination a minor reaction was palpable in 1 of the 10 animals for 2 days. At macroscopical examination of the injection site at two weeks post vaccination pale tissue and some vaccine residues were found. In two animals some necrotic tissue was found, in one of them a micro-abscess (< 2 mm) had been formed. Microscopical examination of the injection site tissue revealed a granulomatous reaction in the muscle and/or connective tissue.

The Committee was concerned regarding the lack of a study proving safety in one week old piglets using a double dose (including twice the amount of adjuvant). However the claim is now amended to exclude vaccination of such young animals.

3. Safety of the repeated administration of one dose

In one experiment, the safety of one dose and the repeated administration of one dose was investigated with two Porcilis Pesti batches both containing 120 EU CSFV-E2 subunit antigen per dose of 2 ml. For each batch a group of five 7 week old piglets was vaccinated twice intramuscularly with one dose (2 ml) at an interval of 4 weeks (basic vaccination scheme). No systemic reactions were observed. Body temperature stayed within normal limits (group means post vaccination between 39.4 - 40.4 °C compared to group means of 39.5 - 40.0 °C during the 5 days prior to vaccination). However, in 2 of the 5 piglets of group A, body temperatures as high as 42.1 °C were recorded 4 hours post second vaccination. Body weight gain from first vaccination until 14 days post second vaccination was 236 g/day on average. No reactions were palpable after the first vaccination. After the second vaccination a minimal although not painful reaction was present in the 5 animals. In one animal a small reaction (size of a hazelnut) was observed, which was inflamed for one day.

In a second experiment safety of one dose and the repeated administration of one dose was investigated with Porcilis Pesti batch 77335 containing 210 EU CSFV-E2 subunit antigen per dose of 2 ml. A group of eight 8 week old piglets was vaccinated twice intramuscularly with one dose (2 ml) at an interval of 4 weeks (basic vaccination scheme). Four similar pigs were kept as unvaccinated controls. No systemic reactions were observed. Body temperature stayed within normal limits. No local reactions were observed.

Conclusion on the observed local and systemic reactions

Local reactions at the injection site, as determined by palpation, were seen in some animals, the frequency varied per experiment ranging from 0 to 50%. If present, they were minor and disappeared within a few days. At post-mortem the injection site was characterised by a slight discoloration and some vaccine residue. On some occasions, micro abscess formation or small amounts of necrotic tissue were found. Microscopically, the injection sites were characterised by a granulomatous reaction with infiltration of macrophages and polymorphonuclear giant cells and cyst formation. The findings at the injection site were in line with what may be expected after a vaccination with a water-in-oil emulsion formulation and the irritation can be considered mild.

No systemic reactions were observed apart from a rise in temperature in some vaccinates. The weight gain was normal.

The CVMP nevertheless requested additional data and requested microscopical examination after experimentation with the recommended dose (120 EU) in 6 week old piglets. Furthermore, since the original claims included vaccination at a younger age than 6 weeks, more safety data were requested regarding the youngest animals claimed by the Applicant.

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New safety experiments were provided in one-week-old and in three-week-old piglets with vaccine batch 801402.1 containing an antigen content of 295 EU per dose of 2ml. In the three –week old piglets the first vaccination was done with an overdose (4 ml) followed 2 weeks later by one dose (2 ml). Ten pigs were vaccinated with Porcilis Pesti; 4 pigs were injected with an aqueous diluent to serve as placebo injected controls. In the one week old piglets essentially the same experimental design was followed with the exception that for animal welfare reason no double dose was given at one-week of age. Instead, the recommended vaccination schedule was applied i.e. two times a single dose 4 weeks apart.

In none of the pigs used in the two studies were adverse systemic or local reactions observed. Approximately 2 weeks after the overdose injection, in about half of the pigs in the 3 week old group nodules (diffuse, 2-5 cm in diameter), were palpable at the site of injection. These reactions were painless and transient in nature. After the repeated one dose injection similar reactions were observed in 2 animals.

In the one week old piglets no local reactions were observed after the first vaccination. After the second vaccination in most animals a similar reaction was observed as described in the 3 week old piglets. Histological examination revealed mainly microscopic granulomatous lesions, vacuoles and cysts. These reactions are characteristic for an oil emulsion vaccine and are due to the adjuvant component.

In the 3 week old piglets study, local reactions were observed at the injection site at the time of the second injection two weeks after the injection of an overdose. On day 14 a local reaction was palpable in three of the ten animals although no local reactions were palpable in the 13 days before. The water/oil emulsion, might explain the slow development of the observed reaction. As this phenomenon was not observed in animals vaccinated for the first time with a single dose, it was concluded that the administration of the overdose of the w/o vaccine might be responsible for the observed late reaction in some of the animals.

The first draft SPC was amended accordingly to indicate under section 5.3 (Undesirable effects (frequency and seriousness)) that local and in most cases transient swelling at the injection site can occur up to 4 weeks after vaccination and that abscesses can be observed at the injection site. In addition, since data were not presented to indicate the outcome of carrying out both inoculations at one site, a sentence was added advising that the second vaccination be carried out away from the site of the first inoculation. In section 5.8 (overdose) it was included that following administration of an overdose pronounced reactions may be observed.

Examination of reproductive performance

In a field trial 71 sows in all stages of reproductive cycle were used. No abortions were recorded, no effect of vaccination was seen on the number of live born piglets or the number of still borns (see below: Safety field trial in sows).

However, the field study in sows was performed with an antigen dose (43 EU) lower than the recommended dose (120 EU). Therefore the CVMP requested the safety of the administration of the recommended dose in pregnant sows should be demonstrated.

In response the Applicant provided two more studies in pregnant sows, one at the National Veterinary Research Institute in Pulawy, Poland and one at Intervet International B.V., Boxmeer, the Netherlands. Both studies were performed with vaccine specifically formulated for this purpose so that it contained an antigen content of 295 EU per dose of 2ml. Essentially, the experimental design of both studies was similar. Three groups each of 5 to 6 gilts were synchronised and inseminated. In different stages of gestation (early, mid, late) a group was vaccinated with Porcilis Pesti twice two weeks apart. In the Boxmeer trial, the first vaccination was given as a single dose, the second as an overdose (4 ml). In the Pulawy trial

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just the opposite schedule was used i.e. an overdose followed by a single dose. In the Boxmeer trial, 4 gilts were injected with an aqueous diluent to serve as controls and to provide reference data for the off-spring. In none of the gilts used in the two studies were adverse systemic or local reactions observed. No clinical signs or an effect on rectal temperature were observed either. At Pulawy no local reactions at all were observed. In Boxmeer, a minor reaction was noted for 1-3 days after the overdose vaccination (diffuse, 0.5-2 cm in diameter). In both trials no effect on the duration of the gestation or on the progeny was observed.

The CVMP agreed that the vaccine is safe for use in gilts and sows during all reproductive stages.

Examination of immunological functions

There are no data suggesting a negative influence on the immune response of the vaccinated animal or of its progeny.

Study of residues

Liquid paraffin and polysorbate 80 and Sorbitan mono-oleate are on the annex II list of EC Regulation 2377/90.

Traces of gentamicin may be present as a remnant for antigen production. Gentamicin is classified in Annex III. However, provisional MRLs elaborated for pigs range from 100 up to 1000 µg/kg and significant exposure following vaccination is therefore not expected.

In conclusion, a withdrawal period is not considered necessary.

Interactions

Since interactions with other products have not been investigated, the Applicant recommends that the product should not be used within 2 weeks before or after parenteral vaccines.

FIELD STUDIES

Two safety field trials have been performed, one trial in finishing pigs and one trial in sows irrespective of their reproductive cycle. In the field trial in finishing pigs special attention was paid to local reaction at slaughter. In the field trial in sows special attention was paid to the possible influence on reproductive performance.

Safety field trial in finishing pigs

Pigs on 4 commercial swine farms in Germany were assigned to one of two treatment groups; Porcilis Pesti or control. A total of 110 pigs were vaccinated twice intramuscularly in the neck with 2 ml Porcilis Pesti, batch 27367 containing 43 EU of CSFV-E2 antigen per dose. The control group (56 pigs) were treated intramuscularly with 2 ml phosphate buffered saline. The first vaccination was administered at an age of 8-12 weeks, the second vaccination was administered 4 weeks later.

After the first vaccination a reduced feed intake and/or tendency to lie down was observed in 6 vaccinated pigs (5.5%) and in 3 controls (5.4%). After the second vaccination this was seen in 3 vaccinates (2.8%) and 2 controls (3.6%). On one farm all pigs (controls and vaccinates)

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coughed on three consecutive days due to bad ventilation. All observations are considered not to be vaccine related. During the 14-day observation period 3 control pigs died, one of rectal prolapse and two due to gastric ulcers. Also 3 vaccinates died, two due to gastric ulcers and one due to endocarditis. Two more vaccinates died before slaughter. Therefore the mortality rate was similar in the vaccinates (4.5%, n = 5) and in the controls (5.4%, n = 3). No significant difference in body temperatures between the groups was observed. The average daily weight gain in the vaccinates did not differ significantly from that of the controls ($p = 0.8$).

After the first vaccination one vaccinate showed a small painless swelling at 4 and 24 hours post vaccination. After the second vaccination mild local reactions were seen in 14 vaccinates (13%). In all 14 cases except one, the local reaction disappeared within 1-3 days. No local reactions were observed in the controls. At slaughter the injection site was inspected in 89 vaccinates and in 44 controls. In 73 (82%) of the Porcilis Pesti vaccinated pigs, signs of an injection site reaction were observed. The majority of these animals had a discoloration <2.5 cm in diameter. Haemorrhages, oedema and residues of the vaccine were not found. In 23 animals (26%) sporadic traces of necrosis were observed and minor calcification was observed in 19 animals (21%). In 43 animals, one or more micro-abscesses (<2 mm diameter) were observed at the site of injection. In 3 pigs (3%) a single abscess and in 4 pigs (4%) several abscesses >2 mm were found.

Safety field trial in sows

Breeding sows on three commercial swine farms in Germany were randomly assigned in the ratio 2:1 to one of two treatment groups: Porcilis Pesti or control. A total of 71 sows were vaccinated twice intramuscularly in the neck with 2 ml Porcilis Pesti, batch 27367, containing 43 EU of CSFV E2 antigen per dose. The control group (36 sows) were treated intramuscularly with 2 ml phosphate buffered saline. The sows were vaccinated twice with an interval of 4 weeks irrespective of their reproductive status. Of the 107 sows involved, 27 were not pregnant, 27 between 0-40 days of gestation, 27 between 41-80 days of gestation and 26 more than 81 days in gestation.

Both after the first and the second vaccination one vaccinate and one control sow showed a reduced feed intake and/or signs of disease probably related to M.M.A. (mastitis, metritis, agalactiae syndrome), farrowing or oestrus, which lasted 1 to 3 days. In one herd a reduced feed intake was observed at 1 hour after vaccination both in vaccinated and in control sows. One vaccinated sow died after the first vaccination (pneumoniae, cystitis and abortion), one after the second vaccination (overloaded stomach). Both cases were not considered vaccine related. At no time was a significant difference in body temperatures observed between the groups.

The occurrence of local reactions was only reported from one of the three herds involved in the trial. After the first vaccination three vaccinates (4.2%) showed a small non-painful swelling or groups of inflammation on the day of first vaccination. The reaction disappeared within 24 hours. After the second vaccination two sows (2.8%) had a similar type of reaction that disappeared within three days. One sow showed a larger reaction (5-10 cm in diameter) that remained present during the 14-day observation period.

No abortions were recorded. One sow died of pneumoniae and cystitis. No effect of vaccination on the reproductive performance was observed. The number of stillborns was 1.2 ± 2.9 in the group of vaccinates and 0.8 ± 0.8 in the controls. The number of stillborn piglets was unaffected by vaccination, but appeared to be higher in large litters of more than 15 piglets per sow in one herd.

The field trials confirm the results obtained in the laboratory safety experiments and show that

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the product is safe for the target animal (pigs from 6 weeks of age onwards) and in the most sensitive category of animals (pregnant sows).

Furthermore, it was remarked that both studies have been performed with a lower antigen content (43 EU) than the recommended vaccine dose. In the field study in finishing pigs, local reactions occurred in 82% of the animals.

ECOTOXICITY

Following the guidance for the Environmental risk assessment for veterinary immunologicals (EMEA/CVMP/074/95) the possible ecotoxicity of using Porcilis Pesti was evaluated.

Direct exposure of the environment to the product does not take place. Any unused or waste material does not pose any environmental risk, but should nevertheless be disposed by the appropriate channels. As no live micro-organisms are present in the product, hazards and risks from the active ingredient are likely to be negligible. Toxic effects of the product compounds or of excreted metabolites are considered not to present a hazard.

Assessment of the risk to the environment

Hazard identification:

The active ingredient in the product, the CSFV-E2 subunit antigen, is produced in the baculovirus expression system. The antigen harvest may contain baculovirus as a remnant of production. To inactivate this baculovirus, the harvest is treated with 0.5% BPL (β - propriolactone) for 3 hours at 26-30 °C. If the inactivation process is not performed properly, Porcilis Pesti can contain live baculovirus. If the hydrolysis of the BPL is not complete, the vaccine may contain residual BPL. None of the other components of the vaccine or their metabolites, even if excreted, can be considered to be an environmental hazard.

Assessment of likelihood:

The chance that the vaccine will contain live baculovirus is nil. The inactivation procedure is validated, inactivation takes places within 67% of the time allowed for inactivation. Furthermore, inactivation is confirmed by the inactivation control test (on antigen and final bulk). BPL residues will not reach the environment, as (i) hydrolysis will be complete when the product is used (several weeks after BPL treatment), and (ii) once injected hydrolysis at 37 °C will be even faster.

Assessment of the consequence: negligible

Assessment of level of risk: negligible

Assessment of the overall risk to the environment: effectively zero

No second phase evaluation is considered necessary.

The CVMP concluded that the absence of infectious baculovirus has been shown by a confirmatory test in the finished product (see part II.). Therefore, the infection risk for insects in the environment is negligible.

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Risks foreseen for the operator and other animals

Risk to the operator

The baculovirus used to produce the subunit antigen in the product is inactivated and the product itself is sterile, hence from biological point of view no risk to the operator exists. The product does not contain toxic excipients. Liquid paraffin, sorbitan oleate and polysorbate 80 are on the Annex II of EC Regulation 2377/90. It can be concluded that also from this point of view no risk to the operator exists, and that, no withdrawal period is necessary. The product has to be administered by injection so, there is always a risk that the operator accidentally injects himself. Since the vaccine is oil-based, this means a risk and therefore the leaflet contains instructions how to act in the case of self injection.

Risk to other animals

The baculovirus used to produce the subunit antigen in the product is inactivated, so no risk of spread to other animals exist. The risk for species other than the target animals is negligible because the vaccine has to be administrated by injection to each individual animal.

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4. OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY TRIALS

INTRODUCTION

Relevance of the data

In the efficacy section of the dossier, four vaccination/challenge experiments have been presented. The main objectives of these experiments were:

- to establish the minimum effective antigen dose (43 EU CSFV-E2 subunit antigen) and the optimal vaccination scheme,
- to establish the duration of immunity (6 months with a vaccine containing the minimum effective antigen dose),
- to establish the onset of immunity (2 weeks post second injection of the basic vaccination).

Although there is a variation in the antigenic mass content of the batches used, they are all relevant for the establishment of the efficacy of the product because they all have the final product formulation.

GENERAL REQUIREMENTS

In the vaccination/challenge experiments pigs ranging from 7 to 9 weeks were used and the recommended intramuscular route of administration was applied. In all experiments but one, the animals were vaccinated according to the recommended vaccination scheme involving two intramuscular injections with a single dose of 2 ml at approximately a 4-week interval. A group of animals in one experiment were vaccinated only once with a high antigenic mass vaccine (210 EU). At first, the dossier did not contain data obtained in animals with maternally derived antibodies (MDA) but on request of the CVMP the data were provided. The onset of immunity was studied in piglets of 7 weeks of age according to the schedule of administration. Duration of immunity was evaluated with a lower antigenic mass (43 EU) and the results support those expected with the recommended antigen amount (120 EU).

LABORATORY TRIALS

EFFICACY OF THE VACCINE

In all experiments but one, the animals were vaccinated according to the recommended vaccination scheme involving two intramuscular injections with a single dose of 2 ml at approximately a 4-week interval. A group of animals in one experiment were vaccinated only once with a high antigenic mass vaccine (210 EU).

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ANTIGENIC DOSE AND VACCINATION SCHEME

In relation to the claim made by the Applicant, an animal is considered protected if it does not become viraemic and/or if infectious virus is not recovered from nasal swabs.

In a first vaccination-challenge experiment, the minimum effective antigenic dose was established. Three groups of five 9-week-old piglets were vaccinated intramuscularly in the neck with 2 ml of Porcilis Pesti formulations containing 43, 15 or 5 EU of CSFV-E2 antigen per dose of 2 ml. The pigs were vaccinated twice with an interval of 4 weeks. At the same time a control group of 5 pigs were treated with 2 ml phosphate buffered saline. Approximately 5 weeks (34 days) after the second vaccination all pigs were challenged intramuscularly with a lethal dose (± 1000 LD_{50}) of the heterologous CSFV strain Alfort 187.

The serological response to the vaccinations was monitored with an E2 antibody specific ELISA test and/or a virus neutralization test. Blood samples were taken the days of first and second vaccination, 4 weeks post vaccination and then 4, 8, 12 and 18 days post challenge.

The protection against challenge was judged by monitoring clinical symptoms, occurrence of death, body temperature, body weight, blood cell counts and the occurrence of viraemia. All the surviving pigs were killed after the observation period of 18 days and post-mortem examination was performed on all animals.

The challenge results show that at antigen levels of 5 and 15 EU of CSFV-E2 antigen a partial protection was obtained and that an antigenic dose of 43 EU prevented viraemia in all five piglets. In both groups vaccinated with 5 and 15 EU one animal died due to causes not related to CSF challenge on day 7 or day 9 post challenge (volvulus in one animal and *Pasteurella. multocida* infection in the other one). A decrease in the WBC counts was recorded in all animals of the group vaccinated with 43 EU 4 days after challenge. However, viraemia tests were negative.

In the duration of immunity experiment it was shown that vaccine formulated at 43 EU per dose protected pigs for a duration of 6 months thus confirming that 43 EU of CSFV-E2 antigen per dose was an effective dose.

The Applicant has also investigated in another experiment whether a further increase to 210 EU CSFV-E2 antigen per dose was advantageous both with regard to efficacy and/or the vaccination schedule. A group of 8, eight-week-old pigs were vaccinated intramuscularly twice at an interval of 4 weeks with Porcilis Pesti containing 210 EU of E2 antigen per dose (group A). A second group of 4 pigs (group B) were vaccinated only once at an age of 11 weeks. The third group (group C) of 4 animals were vaccinated twice with a placebo vaccine at the same time as group A, and served as challenge controls. Groups A and C were challenged 4 weeks after the second vaccination, group B at 5 weeks after the first and only vaccination. All animals were challenged intramuscularly with a lethal dose (± 300 LD_{50}) of the heterologous CSFV strain Alfort 187. The observation period was 16 days for all animals in groups A and C and for one animal in group B. The observation period for the remaining 3 animals in group B was extended to 30 days. The serological response to vaccination and challenge was monitored in all 3 groups with the virus neutralization test (VN), with an antibody specific ELISA test for E2 and E^{RNS}, and with an antigen specific ELISA test for E^{RNS}. The protection against challenge was judged by monitoring clinical symptoms, occurrence of death, body temperature, body weight, blood cell counts, and the occurrence of viraemia and virus excretion. Moribund pigs as well as the pigs surviving during the observation period were killed and post-mortem examination was performed.

All 4 controls displayed severe clinical signs of CSF and were killed in moribund state at 11 to 16 days post challenge. All animals in group A, vaccinated twice, were fully protected

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against challenge, both with respect to clinical signs and to viraemia. Complete prevention of challenge virus replication could not be obtained as shown by the increase in the VN titres at day 16 post challenge and the appearance of antibodies against E^{RNS} in the serum. All animals in group B, vaccinated only once, survived the challenge. Despite having relatively high VN titres at challenge (6.5 log2 on average) these animals were not fully protected. Some animals showed signs of CSF, but much less than seen in the controls. Two of the four animals were found to be viraemic at a low level for a short period. One animal showed typical CSF lesions at post-mortem. However, virus excretion was not detected in the nasal fluid of any animal.

It can be concluded that the standard antigen dose as selected by the Applicant (120 EU per dose of 2 ml) provides protection since the data obtained in the dose finding study and in the duration of immunity study with vaccine formulated at 43 EU per dose show a high degree of protection, both with regard to clinical signs and challenge virus replication. A further increase to 210 EU per dose was not beneficial, neither with regard to the prevention of a take of the challenge virus, nor with regard to the vaccination schedule.

ONSET OF IMMUNITY

In one experiment, 2 groups each of 5 seven-week-old pigs were vaccinated intramuscularly twice at an interval of four weeks with the Porcilis Pesti containing 122 EU CSFV-E2 antigen per dose. A group of two similar pigs, not vaccinated, served as challenge controls. Two weeks after the second vaccination all pigs were challenged intramuscularly with a lethal dose (± 300 LD_{50}) of the heterologous CSFV strain Alfort 187. The observation period was 30 days post challenge to collect additional serological data after challenge. The serological response to vaccination and challenge was monitored in all 3 groups with the virus neutralization test (VN), with an antibody specific ELISA test for E2 and E^{RNS}, and with an antigen specific ELISA test for E^{RNS}. The protection against challenge was judged by monitoring clinical symptoms, occurrence of death, body temperature, body weight, blood cell counts, and the occurrence of viraemia and virus excretion. Moribund pigs as well as the pigs surviving the respective observation periods were killed and post-mortem examination was performed.

Both control pigs showed clinical signs of CSF including high temperatures from day 5 onwards. By day 5 post challenge both became viraemic and virus was recovered from nasal swabs. The controls were killed in a moribund state at eight days post challenge. The tonsils, spleen and lymph nodes were found positive for virus. In contrast, all 10 vaccinates were well protected against the challenge given at 2 weeks post the basic vaccination. The only effect observed was a minor effect on weight gain in 2 of the 10 vaccinated pigs. No viraemia was detectable and no virus could be isolated from nasal swabs taken at 8 intervals during the observation period of 30 days. Post mortem examination did not show any signs of CSF and no virus was found in homogenates of the tonsils, spleen and lymph nodes.

DURATION OF IMMUNITY

In the duration of immunity experiment, two groups of ten 7-week-old piglets were vaccinated intramuscularly with a dose of two ml. All animals were vaccinated twice at an interval of 34 days. As controls, 2 groups of 5 animals were injected with phosphate buffered saline. Three and six months after the second vaccination, respectively, pigs were challenged intramuscularly with a lethal dose (± 300 LD_{50}) of a heterologous CSFV strain, Alfort 187.
The antibody response was monitored using a specific ELISA test for E2 antibodies or the virus neutralization test. Blood samples were taken the days of vaccinations and of challenge. After the challenge, blood samples were taken after 4, 8 and days in the group challenged after 3 months and only after 16 days in the other challenge group. Protection against challenge was judged by monitoring clinical symptoms, body temperature, occurrence of death, body weight gain, number of thrombocytes, lymphocytes, leucocytes, and occurrence of viraemia. All surviving pigs were killed after the observation period of 16 days and post-

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mortem examination was performed on all animals.

At both challenge intervals (respectively 3 and 6 months post vaccination) all controls showed clinical signs of CSF including high temperatures at 2 to 3 days post challenge. A marked decrease in blood cell counts and the presence of viraemia were demonstrated in all controls. The controls were killed in a moribund state at seven to eleven days post challenge. In contrast, all vaccinates were protected against challenge both at 3 and 6 months post vaccination. There were only mild clinical symptoms probably associated with the challenge in some animals at approximately 4 days post challenge. A slight temperature rise was seen in some animals as well as a temporary influence on blood cell counts and body weight. All vaccinates survived the challenge and no viraemia was detectable.

SEROLOGICAL DATA OBTAINED IN THE VARIOUS EXPERIMENTS

In the antigen dose finding study a relationship between antigen dose, group mean VN antibody titre and the level of protection was observed. Increasing levels of antigen correlated with higher group mean VN antibody titres and with increasing levels of protection. The results obtained with the 120 and 210 EU batches support the positive correlation between antigen dose and group mean VN titres although it seems that a plateau is reached at a group mean titre of approximately 7.5 $\log_2$ VN.

It is clear, however, that the protective immunity is not only based on the neutralising antibody titres but that other, yet unknown, mechanisms also play a role in the protection. In the experiment where pigs were only vaccinated once with a high antigenic mass vaccine, the animals were found to be not fully protected despite having relatively high VN titres at point of challenge (6.5 $\log_2$ on average). In the duration of immunity study, 5 pigs with VN titre in the order of 2 to 4 log2 were found to be protected.

HORIZONTAL SPREAD AND INTRAUTERINE INFECTION

Both aspects were not addressed in the original dossier. Therefore the CVMP requested that a new experiment with sentinel animals after nasal challenge should be performed to study the potential spreading of wild virus. During this experiment, multiplication of the challenge virus in vaccinated pigs should be investigated within the first week after the challenge.

A new experiment was provided in which the spreading from a naive animal, infected with a highly virulent strain of CSFV, to Porcilis Pesti vaccinated in-contact animals was investigated. Fourteen out of fifteen eight-week-old pigs were vaccinated with Porcilis Pesti containing 120 EU per dose of 2 ml. Vaccination was done using the recommended vaccination scheme i.e. 2 vaccinations 4 weeks apart. Fourteen days after the booster vaccination the non-vaccinated pig was isolated and challenge infected by intramuscular and intranasal route with 2 ml of the high virulent CSFV Behring strain containing 3 $\log_{10}$ $TCID_{50}$ per ml. One day after the challenge infection the "shedder" pig was reintroduced to the vaccinated sentinel pigs.

From all animals blood samples were collected at 2 days intervals starting at 1 week before the challenge infection up to 7 weeks post challenge. Serum samples were tested for antibodies to the CSFV-E2 antigen by Elisa and for virus neutralising antibody titres. Virus isolation was attempted from serum, plasma, heparanised blood and blood clot.

All vaccinated pigs had responded with adequate VN antibody titres (range 5.9-9.9 $\log_2$ VN) at point of introduction of the challenge infected shedder pig. The challenge infected pig died at day 6 post infection, indicating a highly effective challenge infection. All vaccinated sentinel pigs survived and no clinical signs occurred. Eleven out of 14 vaccinated pigs became simultaneously infected by the shedder animal as evidenced by the clear anamnestic

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response at day 10-13 as observed in the VN test and the positive reaction at day 17-20 in the E^{RNS} marker test. One vaccinated animal showed a clear anamnestic response in the VN test by day 17 and was found positive for antibodies to E^{RNS} by day 22. The short delay as observed in this animal is possibly due to the high level of neutralising antibodies at point of challenge (9.9 $\log_2$) and thus the anamnestic response is very probably also the result of primary infection by the in-contact animal. Four out of the 12 vaccinated and infected animals were found to be viraemic at one sampling point i.e. at day 10. The remaining two vaccinated animals did not show the anamnestic response in the VN assay and they remained E^{RNS} antibody negative for the total test period of 49 days. Apparently, the twelve primary infected animals were not able to infect these two penmates.

The results of this experiment show that Porcilis Pesti vaccinated animals may become infected when they are in close contact with a pig infected with a high virulent strain of CSFV and that viral transmission to vaccinated penmates is prevented.

A new study was provided in pregnant sows to demonstrate that the vaccine is able to prevent intrauterine infection. A group of 10 gilts was vaccinated as recommended (2 vaccinations 4 weeks apart) with Porcilis Pesti containing 120 EU per dose of 2 ml. Four gilts were injected with an aqueous diluent to serve as controls. Approximately 5 weeks after the second vaccination all gilts were inseminated. In the middle of gestation (day 65 of pregnancy) all gilts were infected intranasally with 6.9 $\log_{10}$ $TCID_{50}$ of the CSFV strain Glentorf. This strain has been described to be particular useful in reproducing the intrauterine infection.

Serum samples were taken to monitor the serological status pre and post vaccination and post challenge. Virus isolation attempts were done at five and nine days post challenge from nasal swabs and white blood cells. At the end of the gestation all gilts were slaughtered, the foetuses were collected and their number and condition recorded. Autopsy was done on the gilt and their offspring. Samples, including white blood cells, tonsils, lymph nodes and bone marrow were collected for virus isolation attempts.

One week after the second vaccination all vaccinated gilts had responded adequately to the vaccination in that they all had developed VN titres of $\geq$ 5.0 $\log_2$ (average 6.5). At challenge, 3 months later, the titres had slightly decreased to an average of 5.2. The four control gilts were found negative. By day nine post challenge all vaccinated gilts showed a clear anamnestic response, indicating that the challenge infection was effective. The four control animals reacted with a primary response. The results of the E2 and E^{RNS} antibody Elisa tests were in line with expected values. Post vaccination only E2 antibodies were detected in all the vaccinates. Post challenge all animals became positive for antibodies to E^{RNS}. This confirms the marker property of the vaccine. No challenge virus could be isolated from any of the vaccinated gilts whereas all four controls became viraemic by day 5 and two had virus in their nasal swabs. The challenge infection had no obvious effect on the pregnancy of these animals in that no abortion occurred. However, at post mortem a clear effect could be seen. Only 25 % of the progeny of the controls were found alive. 52% was found dead and 23% were mummified. In the vaccinates the percentages were 90%, 2% and 8% respectively.

The autopsy did not show signs of CSF in any of the gilts nor was it possible to demonstrate the presence of viral antigen in serum or to isolate virus from any of the organs, including a persistent infection of the bone marrow. In the progeny of the control sows signs of CSF were found in all four litters (100%). In contrast, from the vaccinates only one litter out of ten showed signs of CSF (10%). These results were in all cases confirmed by virus isolation from organs and blood.

In conclusion, Porcilis Pesti protected 90% of the litters from viral infection when sows are challenged at mid gestation with a strain of CSFV described to be suitable to reproduce the intrauterine infection.

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The CVMP initially questioned, whether scientifically, the use of vaccinated sentinels instead of true sentinels provided full insight in to the potential spreading of the wild virus. Following the oral explanation however, the CVMP agreed that the above trial mimicked sufficiently the field situation which would occur when ring vaccination would be performed, and furthermore concluded that this trial provided adequate insight into the potential spreading of the wild virus.

Additional information on efficacy:

The CVMP requested comments from the Applicant on the protective effect of maternal antibodies and the potential interference on vaccination. Piglets were collected from the sows used in the experiment to study safety during pregnancy and which were vaccinated with a double dose followed by a single dose with a vaccine containing a very high antigenic mass (295 EU per dose of 2 ml). Groups of piglets were vaccinated with Porcilis Pesti containing 120 EU per dose of 2 ml according to the recommended vaccination schedule. The first group was vaccinated at 5 and 9 weeks, the second group at 7 and 11 weeks and the third group at 9 and 13 weeks of age. The fourth group was not vaccinated to serve as controls. The development of VN antibody titres was used as the criterion for break through. The control group showed a linear decline in $\log_2$ VN serum titres from 3 weeks of age (first sampling point) up to 9 weeks of age at what time point they reached the detection limit of the VN assay. All three vaccinated groups showed a good take of the vaccine regardless of the age at which the vaccination was given. Maternal derived antibodies did not interfere with vaccination.

The youngest age recommended for vaccination was considered to be 5 weeks. Indeed, in 2 experiments, the vaccine has been shown to elicit VN antibody titres which correlate with protection when the vaccine is given at an age of 5 weeks. Until efficacy data have been generated in younger piglets, no efficacy will be claimed in pigs younger than 5 weeks.

Marker test system

The RNA genome of CSFV contains one large open reading frame encoding a polyprotein of about 3900 amino acids. This polyprotein contains the viral structural and nonstructural proteins. The viral structural proteins including the capsid protein and the envelope proteins E^{RNS}, E1 and E2 are located in the N-terminal part of the polyprotein. The nonstructural proteins including the serine protease NS3, NS4A/B and NS5A/B are located in the C-terminal part of the polyprotein.

Pigs in a herd infected with CSFV develop, amongst others, antibodies against E^{RNS} and E2. As Porcilis Pesti contains only the E2 subunit antigen of CSFV, animals in a vaccinated herd will only develop antibodies to the E2 antigen of CSFV. Thus, by testing pigs for the presence of antibodies to E2, pigs which have been in contact with this antigen (either by vaccination or by infection or both) can be identified. By testing for the presence of antibodies to E^{RNS}, a herd infected with CSFV could be distinguished from a herd vaccinated with Porcilis Pesti once an appropriate test system becomes available.

For proper function, the following aspects are essential in such a marker system:

1. To distinguish vaccinated pig herds from non-vaccinated pig herds, the marker vaccine must consistently elicit antibodies to the subunit antigen it contains (E2). It is well established for CSFV that the viral envelope proteins ^{ERNS} and E2 are immunodominant. It is therefore logical to develop a vaccine/marker test system on the basis of these two antigens. Since antibodies to especially E2 are strongly neutralizing, which is of relevance for protection but not essential for the marker test, the Porcilis Pesti vaccine contains the

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E2 subunit antigen. In the vaccination experiments performed by the Applicant all vaccinated animals tested had developed antibodies to the E2 antigen, even all the animals vaccinated with the 5 and 15 EU vaccines in the dose finding study.

2. To avoid false-positive results the marker vaccine should not induce antibodies to the antigen used to distinguish between vaccinated and infected pig herds (E^{RNS}). In the vaccination experiments performed by the Applicant none of the vaccinated animals tested had developed antibodies to the E^{RNS} antigen.

3. To avoid false-negative results infected pig herds should elicit antibodies to the antigen used to establish an infection, in this specific case, antibodies to E^{RNS}, even when vaccinated. In both vaccination-challenge experiments in which the development of antibodies to the E^{RNS} antigen was monitored, antibodies to the E^{RNS} antigen became detectable by day 9 to 16 post challenge. From day 16 to 21, 40-75% of the pigs were found positive which is sufficient to establish an infection in a herd (decisions are taken on herd level, not on individual pigs). A possible explanation for the fact that not all animals developed anti-E^{RNS} antibodies is that the vaccine may induce an immunity which is close to a level which prevents multiplication of the virus however insufficient to elicit a rapid' primary anti-E^{RNS} response. Most animals showed a booster response except in 2 animals which both had a very high neutralising antibody titre at point of challenge and probably had an unusually strong immunity.

4. Preferably, to avoid false positive results, the distinguishing antibody should not be elicited by other viruses capable of infecting the target animal or a marker test kit should be made virus-type specific. The related ruminant pestiviruses are capable of infecting pigs and thus may elicit an anti-E^{RNS} antibody response, which in conjunction with CSF would represent a false positive result. When testing 644 sera obtained from slaughter houses in Switzerland with a E^{RNS} antibody ELISA test kit, the specificity was found to be 97.8%. The anti-E^{RNS} Elisa test currently used is not specific for anti CSFV-E^{RNS} but it detects antibodies against E^{RNS} of different pestiviruses. Thus, it can not be excluded that some of the 2.2% positive results recorded in the pig sera are caused by ruminant pestivirus infections in farms housing both pigs and cattle. In such a case this is a specific but false positive anti-E^{RNS} reaction because they are not due to a CSFV infection.

In conclusion, the marker property of the vaccine appears to function adequately. Vaccinated animals only develop antibodies to E2 whereas pigs in an infected herd (whether vaccinated or not vaccinated) will develop antibodies to, amongst other antigens, E2 and E^{RNS}. Thus, vaccinated herds can be distinguished from infected herds by using proper diagnostic tools.

A further challenge trial in suckling piglets was requested by the CVMP to prove efficacy in newborn piglets. However these questions were left unanswered and the CVMP agreed with the proposal from the Applicant to change the SPC to the following:

> "Active immunisation of pigs <u>from the age of 5 weeks</u> onwards to prevent mortality and to reduce clinical signs of Classical Swine Fever, as well as to reduce infection with and excretion of CSF field virus."

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FIELD TRIALS

The Applicant was informed that the European Commission and other Member States were not in favour of a field trial. At the European level the Scientific Veterinary Committee of the EU Commission stated that "field experiments with a vaccine during an actual outbreak are not easily envisaged" (document VI/8119/97, Annex 2 of 12-11-1997). In a meeting on 12-02-1998 with representatives of Belgium, Germany, the Netherlands (ID-DLO) and DG SANCO the Applicant was informed that Member States will not allow field trials to facilitate licensing, because of the consequences on trade this might have as a result of Directive 80/217/EEC (Document VI/8119/97).

In conclusion, no field efficacy trial with Porcilis Pesti was performed.

RISK-BENEFIT ASSESSMENT AND CONCLUSION

The data submitted in the dossier, in response to questions, and further provided at the oral explanation confirm the acceptability of the proposed formulation and presentations, the suitability of the specification for the active ingredient, the method of manufacture of the product and the validity of the test methods applied to the product. The stability tests provided for the finished product show that the product is stable for 12 months. The retained in-use shelf life is 3 hours.

The safety documentation showed that a transient hyperthermia may occur after the second vaccination. After administration of the product at the recommended dose, a local and in most cases transient swelling at the injection site may occur up to 4 weeks after vaccination. Abscesses may also be observed at the injection site, and at slaughter, up to 82% of pigs show signs of local reactions. Local reactions can occur as discoloration, necrosis, micro-abscesses or calcification and are microscopically characterized as granulomatous with inflammatory cell infiltration. All those reactions could be ascribed to the adjuvant, more than to the antigen. As local reactions were not systematically more severe after the second vaccination, a sensitization phenomon is unlikely. It is consequently advised to carry out the second vaccination at a different site to the first vaccination. The safety of the administration of the product at the recommended dose has been demonstrated in piglets of one-week, 3 weeks and 6-8 weeks of age. The safety of the product in finishing pigs was partially demonstrated by a study using a lower antigen dose. As no dramatic local or systemic reactions using the recommended dose have been recorded in piglets, no more reactions of a more serious nature than those recorded in piglets are expected in finishing pigs. The safety of the repeated administration of one dose was demonstrated in pregnant sows. As the product will be given by intramuscular route and free of infectious baculovirus, the environmental exposure is negligible. The general safety for animals, human and environment is therefore considered acceptable.

Efficacy trials with Porcilis Pesti were performed in piglets. All the vaccination schemes tested at the different doses (5, 15, 43, 120 and 210 EU) have protected piglets against mortality induced by a CSF challenge. Characteristic lesions for CSF, but no mortality, were only observed in one animal vaccinated only once with 210 EU. Moreover, one injection alone did not protect against viraemia. These observations therefore justify the vaccination schedule of two injections at a 4-week interval. The dose antigen finding study showed that an amount of 43 EU with 2 vaccinations at a 4-week interval was sufficient to protect against viraemia. According to the dose-effect relationship in the VN titres, the choice of the Applicant to increase the amount of antigen is entirely acceptable.

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The Applicant has demonstrated that the onset of immunity occurs 2 weeks after vaccination by performing a challenge test at the appropriate time interval. Moreover, at this time 100% of vaccinated piglets were positive for anti-E2 antibodies. A duration of immunity against mortality is supported for 6 months as shown by a challenge at this time and a 100% serological response for anti-E2 antibodies.

Vaccination appears to protect against viraemia and virus excretion in the nasal swabs. However, vaccination does not protect against virus replication as shown by increases in VN titres and E^{RNS} antibodies after challenge, the latter being necessary for diagnostic differentiation.

All the studies demonstrate a protection against mortality induced by CSFV for piglets 7-9 weeks of age. Since the vaccine has been shown to elicit VN antibody titres which correlates with protection when the vaccine is given at an age of 5 weeks, the claim for this minimal age for vaccination can be recommended.

The conclusions of the studies performed in piglets could be extrapolated to finishing pigs as the latter are less sensitive than the former.

A challenge performed by the intranasal route in sows showed that the vaccine protected 90% of the litters from viral infection. It has been shown that maternal derived antibodies did not interfere with vaccination.

In conclusion, the efficacy of the products has been demonstrated in preventing mortality and a marked reduction of clinical signs and reduction in CSF virus excretion in weaned piglets.

Based on the original and complementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC.

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