EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines and Product Data Management

Scientific discussion

This module reflects the initial scientific discussion for the approval of ZULVAC 1+8 Ovis (as published in March 2011). For information on changes after this date please refer to module 8.

1. Summary of the dossier

ZULVAC 1+8 Ovis is an aluminium hydroxide saponin adjuvanted vaccine for the active immunisation of sheep in order to prevent viraemia established in animals infected by serotype 1 and 8 of Bluetongue Virus (BTV). The active substance of ZULVAC 1+8 Ovis is the inactivated bluetongue virus serotypes 1 and 8 (BTV1 and BTV8).

ZULVAC 1+8 Ovis was eligible for the centralised procedure under Article 3(2) of Regulation (EC) No 726/2004 as it is an immunological veterinary product for the treatment of animal disease subject to community prophylactic measures.

The benefit of ZULVAC 1+8 Ovis is the stimulation of active immunity in sheep against the bluetongue virus, serotypes 1 and 8. A 2 ml dose of the vaccine is recommended to be administered by subcutaneous route to sheep (including pregnant animals). The basic vaccination schedule consists of one initial injection given from a minimum of 1.5 month of age and followed by a second injection given 3 weeks later. Onset of immunity is 3 weeks after the completion of the basic vaccination course. The duration of immunity of the vaccine is 1 year.

Bluetongue Virus can cause intense disease outbreaks in sheep. Fever is the most usual but not invariable clinical sign. If fever occurs sheep first become pyrexic 4-10 days after infection. The acute form in sheep is usually characterised by pyrexia up to 42°C, depression, emaciation, ulceration of the oral cavity, swollen and sometimes cyanotic tongue, excessive licking movements of the tongue, lameness and abortion. Infection may result in the death of sheep within approximately 8-10 days or in a long recovery period with negative impact on the animals' welfare and growth. Mortality rate in sheep could reach up to 70% in a flock.

Bluetongue serotype 1 has been responsible for outbreaks in the regions of Spain, Portugal and France, whereas, recent epidemics due to Bluetongue serotype 8 have occurred in several countries in Europe such as Belgium, Denmark, France, Germany, Luxembourg, The Netherlands, United Kingdom, Czech Republic, Switzerland, and also Austria, Italy, Spain, Sweden and Norway.

The dossier was reviewed in line with the provisions of Article 39(7) of Regulation (EC) No 726/2004 for an authorisation under exceptional circumstances and the recommendations of the CVMP Guideline on minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against bluetongue (EMEA/CVMP/IWP/220193/2008).

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There was no need for an inspection to take place following the favourable outcome of the last inspection. The presented Pharmacovigilance (PhV) System was considered accurate and overall fulfilled relevant requirements.

2. Quality assessment

Composition

The composition for one dose of ZULVAC 1+8 Ovis of 2 ml is provided in the following table:

Names of ingredients		**Quantity per 2 ml dose**	**Function**	**Reference to standards**
Active ingredients	Inactivated Bluetongue virus (BTV), serotype 1, strain ALG2006/01 E1	RP ≥ 1(1)	Antigen	In-house
	Inactivated Bluetongue virus (BTV), serotype 8, strain BEL2006/02	RP ≥ 1(1)	Antigen	In-house
Constituents of the adjuvant	Aluminium hydroxide hydrated for adsorption	4 mg	Adjuvant	Ph. Eur. 1664
	Saponin	0.4 mg	Adjuvant	In-house
	Thiomersal	0.2 mg	Preservative	Ph. Eur. 1625
Constituents of the excipient	Saline solution	*q.s.* 2 ml	Diluent	In-house

(1) Relative potency by a mice potency test compared to a reference vaccine that was shown efficacious in lambs. The blending of vaccine is based on the infection titres of BTV-1 and BTV-8 ($TCID_{50}$/ml) before inactivation

Container

The vaccine is filled in 25 (10x2ml doses), 100 (50x2ml doses) and 250 ml (120x2ml doses) capacity High Density Polyethylene (HDPE) bottles. Each bottle is closed with a 20 or 32 mm diameter chlorobutyl rubber stopper and sealed with an aluminium cap. Each bottle is individually labelled and packed into a product specific cardboard box. The product is packaged together with a product information leaflet. Before filling the bottles are sterilised by gamma irradiation. Before use, the rubber stoppers are siliconised and steam sterilised. A compliance certificate on the sterilisation method was provided, as well as a detailed description of the silicone oil used as a lubricant of the rubber stoppers.

Development Pharmaceutics

The main characteristics of the Bluetongue virus (BTV) strains from which the vaccine antigens were derived are the following:

- Serotype 1 strain BTV-1/ALG2006/01 E-1: provided by Laboratorio Central de Veterinaria (LCV, Algete, Madrid, Spain). The strain originates from an Algerian outbreak in 2006.
- Serotype 8 strain BEL2006/2: provided by the Belgian Veterinary Health reference laboratory VAR-CODA-CERVA (*Centre d'Etudes et de Recherches Veterinaires et Agrochimiques,* Ukkel, Belgium). The strain originates from a Belgian outbreak in 2006.

For both BTV serotypes, an expert assessment was provided confirming the relevance of the two vaccine virus strains to the current epidemiological situation of BTV infection in Europe.

The two virus strains were propagated in BHK-21 cells for the production of the corresponding master seed virus (MSV). The working seed virus (WSV) is prepared after passages from the MSV. Vaccine antigens are obtained from MSV on BHK-21 cells. Each virus strain harvest is titrated, and before the formulation of the vaccine, the virus strain harvest(s) are inactivated with binary ethylenimine. An inactivation control test is then carried out in order to rule out presence of residual infectious virus particles.

The adjuvant system (aluminium hydroxide and saponin) used in the formulation of the vaccine, was selected according to the encouraging results obtained within the development of ZULVAC 4, an inactivated vaccine against BTV4 which was granted an authorisation by the Spanish Medicament Authorities in August 2007. The selected adjuvant system was the one capable of inducing in the host an immune response able to prevent the viraemia, as well as acceptable general and local reactions from the standpoint of safety. Thiomersal has been used as preservative in multi-dose containers. The saline solution is used as diluent of the antigen, and is added in sufficient quantity (q.s.) to maintain constant the quantity of antigen per dose (2 ml for lambs). A series of preliminary studies were carried out in sheep using experimental batches of a monovalent proprietary vaccine containing a different BTV serotype (i.e. ZULVAC 4), in order to determine the optimal quali-quantitative composition in terms of adjuvants and concentration of vaccine antigen. Based on the results obtained from a list of studies, a concentration of 1x 10^7 $TCID_{50}$ of vaccine antigen (before inactivation) and a quantity of 4 and 0.4 mg/dose respectively of aluminium (ion) and saponin were selected as target concentrations in final batches of ZULVAC 4 vaccine per dose. The information generated from these experiments was also taken into account for the development of other proprietary BTV vaccines, including the one under application, based on the fact that the same process is used for the manufacturing of the vaccine antigen, and the inactivation of BTV-1 and BTV-8 is obtained under the same conditions established for the BTV4 serotype. In addition, the same adjuvant(s) at the same concentration(s)/ dose are used.

The vaccine under application is blended on the basis of the pre-inactivation viral titre of the bulk antigen. This is allowed in the the CVMP Guideline Paper on Minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against Bluetongue (EMEA/CVMP/IWP/220193/2008). To this aim, in order to establish the concentration of the active ingredients to be used in the formulation of the bulk vaccine, a specific dose-response efficacy study (presented in Part 4) was designed in order to test the immunogenicity in 1.5 month old lambs of the current vaccine at different concentrations of inactivated BTV-1 and 8 antigens. According to the conclusions of the study ZULVAC 1+8 Ovis vaccine - when inoculated in 1.5-months-old lambs at a concentration of $10^{6.1}$ $TCID_{50}$/2 ml dose of each serotype - prevented viraemia in all vaccinated animals after a virulent challenge carried out by subcutaneous route with 2 ml of BTV serotype 1 at a concentration of $10^{6.5}$ $TCID_{50}$/ml, and with 2 ml of BTV serotype 8 at a concentration of $10^{6.1}$ $TCID_{50}$/ml.

However, in order to ensure that commercial batches will still meet the final product specification for host animal potency at the end of shelf-life, and taking into account the results obtained from a pivotal efficacy study a batch of ZULVAC 1+8 Ovis formulated on the basis of the pre-inactivation titres of $10^{6.5}$ $TCID_{50}$/2ml dose for both BTV serotypes, was established as the reference batch. A relative potency (RP) of 1 was assigned to this batch, which means that in order to ensure that consistent batches of ZULVAC 1+8 Ovis are released these batches must have a RP$\geq$1 in the validated batch potency test.

The minimum antigen content of $10^{6.5}$ $TCID_{50}$/dose at blending was set for each BTV serotypes. The minimum antigen content of $10^{6.5}$ $TCID_{50}$/dose was set to ensure that commercial batches will still meet the final product specification for host animal potency at the end of shelf-life. The maximum antigen content of $10^{7.0}$ $TCID_{50}$/dose was set taking into account the results of relevant safety studies.

The duration of immunity study, using batches E-51 and E-52 of ZULVAC 1+8 Ovis, supported evidence for a duration of immunity (DoI) for one year.

The physico-chemical controls carried out on the vaccine ZULVAC 1+8 Ovis include the tests indicated in Ph. Eur. monograph 0062. The nature of the container materials and of the closure system were chosen according to Ph. Eur. recommendations for injectable preparations.

Packaging:

The vaccine will be available in 10, 50 and 120 doses presentations, and in the following package sizes:

- HDPE bottles of 25 ml capacity, for a filled volume of 20 ml, equivalent to 10 doses of 2 ml.
- HDPE bottles of 100 ml capacity, for a filled volume of 100 ml, equivalent to 50 doses of 2 ml.
- HDPE bottles of 250 ml capacity, for a filled volume of 240 ml, equivalent to 120 doses of 2 ml.

Conclusions on Development Pharmaceutics

The background information relating to the development of the vaccine, and to the manufacturing process, was satisfactorily addressed. The process is similar to the one used for the production of ZULVAC 8 Ovis/Bovis vaccine.

Method of manufacture

The stages of the manufacturing process were described in sufficient detail. All the operations stated to be conducted in conditions of sterility following established methods or sterile manipulation techniques. The manufacturing process consists of two steps: the production of the two vaccine antigen(s), and the preparation of the finished product. Detailed flow charts of the two manufacturing steps were provided and considered satisfactory. The process starts with the propagation of the WSV and ends with the preparation of the vaccine in bulk, followed by filling and packing of the final product. The production system and control guarantees the traceability of each component during the manufacturing process.

Manufacture of vaccine antigen(s)

The vaccine viruses are grown in BHK-21 cells. A MSV was constituted on BHK-21 cells, and stored frozen prior to vaccine production. The WSV is expanded from the MSV into BHK-21 cells and is stored frozen.

In the antigen production process, the virus vaccine is produced from the WSV by a number of passages into BHK-21 cells.

Manufacture of the inactivated and neutralised vaccine antigen

The final viral suspensions are inactivated with binary ethylenimine. The excess of inactivating agent is then neutralised at the end of the inactivation process. Samples are taken from the inactivated and neutralised antigen to carry out appropriate in process controls. The titre of the antigen before inactivation and the dilution factor that represent the additions in the inactivation and neutralisation processes are taken into account in order to provide an indication of the theoretical titre of the inactivated and neutralised antigen. Provisions in the current legislation allow for the virus titre before inactivation to be considered as appropriate for vaccine formulation. The standard titre is the average of pre-inactivation titres based on experience with antigen bulks. However, for an antigen bulk to be acceptable the virus harvest titre must be between the minimum and maximum as detailed below. The same criterion is also used when calculating a standard batch composition.

The limits set for minimum /maximum "standard" titres of the virus yields were provided.

The quantity of antigen per dose is calculated and adjusted in each produced batch according to the titre of the bulk antigens before inactivation and it is between the minimum quantity used in the efficacy trials ($2x10^{6.5}$ $TCID_{50}$/2 ml dose) and the maximum quantity used in the safety trials ($2x10^{7.0}$ $TCID_{50}$/2 ml dose).

Manufacture of the finished product

The preparation of the finished product was detailed satisfactorily. The bulk vaccine is prepared by blending pre-determined amounts of one or a mixture of several batches of inactivated and neutralised BTV antigens with thiomersal, saline solution and adjuvants. Two alternative processes for blending can be used, a single and a 2-tank manufacturing process respectively. Both are proven to give a homogeneous and consistent final bulk. Consistency data for three consecutive finished product batches (max/min batch sizes, i.e. 120 and 10 doses) manufactured according to the manufacturing process above were provided together with the corresponding Manufacturer's Batch Protocols.

Composition of the batches used in clinical trials

Details of the vaccine preparations used in laboratory trials were provided, including details of the vaccine antigen production and of the manufacturing of the finished product. Overall they were considered appropriate for use in safety and efficacy clinical trials.

Validation studies

A number of studies were presented as part of the validation of the manufacturing process. The majority of the control test methods were already satisfactorily assessed during the central authorisation of the monovalent ZULVAC 8 Ovis and ZULVAC 8 Bovis vaccines and methods were considered as adequately validated. An additional assurance to the validation of the manufacturing process and quality control was provided by the inactivation kinetics study results and the fact that control of a complete inactivation was observed together the *in vivo* batch potency test. The validation of the in *vivo* batch potency test in transgenic mice was performed based on the results obtained from three doses titration studies. The statistical analysis of the results showed that there is a significant dose-response effect (logistic regression and test for linear trend). A correlation between prevention of mortality and content of pre-inactivation virus particles per dose of the vaccine was demonstrated. Finally, the experimental animal model was proven to be sufficiently sensitive to be able to distinguish between standard and sub-standard formulated batches.

Inactivation kinetics

Standard inactivation kinetics studies were conducted in compliance with the Ph. Eur. requirements concerning the time required for the inactivation of the vaccine antigen (not more than 67% of the duration of the inactivation process). Two studies were performed for each BTV serotype. Inactivation kinetics were tested using a BTV suspension with a titre representative of routine production batches (i.e. reference batch), followed by inactivation kinetics of tenfold (10x) concentrated BTV suspension. Based on the results of the second study the maximum pre-inactivation titre was established for each BTV serotype. A similar experimental model was adopted for all studies (production of the reference virus suspension, inactivation, sampling during inactivation process at relevant time points, control of inactivation). Based on the results obtained, the maximum pre-inactivation titre was $10^{8.6}$ $TCID_{50}$/ml and $10^{8.3}$ $TCID_{50}$/ml for BTV-1 and BTV-8, respectively.

The validation of the test for complete inactivation was also provided as part of the in process control tests and the limit of detection were set for BTV-1 and BTV-8 respectively.

Control of starting materials

Starting materials listed in a pharmacopoeia

The following starting materials of biological origin are used in the manufacture of ZULVAC 1+8 Ovis vaccine:

Starting Material	Function	Species
BHK-21 cells (clone 13)	Substrate for the replication of BTV	Baby hamster
Bluetongue virus, serotype 1 (BTV-1)	Master and Working Seed Virus	Bovine
Bluetongue virus, serotype 8 (BTV-8)	Master and Working Seed Virus	Bovine
Bovine calf serum* irradiated	Source of proteins for the cell substrate	Bovine
Glasgow MEM	Cell Culture	Bovine
Trypsin	Trysinisation	Porcine, bovine
Saponin	Component of adjuvant	Vegetable
Microcarriers	Support for BHK cell growth (optional)	Pig skin

* Starting material also listed in Ph.Eur. 2.2.6.2

A detailed description including information on their function, species origin and treatment before use of the starting materials listed above was provided which was considered satisfactory. All of them, besides the BTV serotype 1, were also satisfactorily assessed during the central authorisation of the monovalent ZULVAC 8 Ovis and ZULVAC 8 Bovis.

Active substances

Bluetongue Virus serotype 1 (BTV-1)

The Strain BTV-1/ALG2006/01 belongs to the collection of the Institute for Animal Health (IAH, Pirbright, UK) and its history was provided. BTV Type 1 was identified as BTV by a specific RT-PCR. The place of original sample was Berrouaghia Media, Algeria. It was isolated from a sheep spleen at IAH Pirbright, by inoculation into Chicken Embryonated Eggs (CEE) on 24 September 2006.

A sample of the strain was supplied to Laboratorio Central de Veterinaria, LCV (Spanish BTV Reference Lab, Madrid), Algete, on 30 October 2006. The virus was tested by RT-PCR against Pestivirus, Classical Swine Fever virus, Foot and Mouth Disease virus and Contagious Ecthyma virus with a negative result. In 2007, a sample of the virus was provided by LCV to Fort Dodge Veterinaria, S.A.

The original Master Seed Lot was tested for identity, sterility, absence of *Mycoplasma,* absence of extraneous agents, titration (determination of $TCID_{50}$) with satisfactory results. The WSV stock was prepared from the MSV by means of two passages in BKH-21 cells, according to the method described above. The WSV was prepared under GMP conditions.

In order to determine that the Bluetongue virus WSV is free from contaminants and suitable for the manufacture of vaccine, the following controls are conducted: sterility, absence of *Mycoplasma,* absence of pestivirus, titration (determination of $TCID_{50}$).

Bluetongue Virus serotype 8 (BTV-8)

The vaccine virus strain was isolated from the blood of an infected sheep during an outbreak of BTV in Belgium in 2006 and BTV was confirmed by RT-PCR.

The original Master Seed Lot was tested for identity, sterility, absence of *Mycoplasma,* absence of extraneous agents, titration (determination of $TCID_{50}$) with satisfactory results. The WSV stock was prepared from the MSV by means of two passages in BKH-21 cells, according to the method described above. The WSV was prepared under GMP conditions.

In order to determine that the Bluetongue virus WSV is free from contaminants and suitable for the manufacture of vaccine, the following controls are conducted: sterility, absence of *Mycoplasma,* absence of pestivirus, titration (determination of $TCID_{50}$).

Starting materials of non-biological origin

Details, relevant control tests and certificates of analysis were provided for the following substances and were considered satisfactory:

Starting Material	Function
Bromoethylamine hydrobromide	Inactivating agent
Dimethyl sulfoxide (DMSO)	Cryopreservative

In House preparation of media

The description of constituents (together with information on the quali-quantitative constituents, and shelf life of each preparation), the method of preparation (including sterilisation) and the basic controls carried out during preparation was provided to support the quality of the following media:

Starting Material	Function
Glasgow MEM Culture Medium	Growth medium for BHK-21 cells
Phosphate-buffered saline solution	Formulation of trypsin solution-cells, and formulation of trypsin 0.05% solution
Saline solution	Saline solution is used as antigen diluent, added in quantity sufficient (q.s.) to complete the volume established per dose
Trypsin solution - cells	Formulation of trypsin 0.05% solution
Trypsin 0.05% solution	Trypsinisation of BHK-21 cells
Disodium edetate 5% solution	Formulation of trypsin 0.05% solution
Sodium hydrogen carbonate 7.5% solution	Formulation of trypsin 0.05% solution
Phenol red solution	Formulation of trypsin solution - cells
Sodium hydroxide 1N, 2N and 6N solutions	For pH adjustments
0.1M binary ethylenimine solution	Inactivation of BTV
1 M Sodium thiosulphate solution	Neutralisation of BEI after the inactivation of the BTV
Thiomersal 10% solution	Vaccine preservative
Saponin 1% solution	Vaccine adjuvant

They were considered acceptable.

Packaging:

Tests of compliance with relevant Ph. Eur. requirements were provided and were found satisfactory (see container section).

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

An assessment of the starting materials was conducted in accordance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products (EMEA/410/01-Rev.2), the Position Paper on the Assessment of the risk of transmission of animal spongiform encephalopathy agents via Master seed materials used in the production of veterinary vaccines the and Commission Directive 199/104/EEC.

The assessment conducted demonstrated that the risk of transmission of TSE by the starting materials of animal origin used in the manufacture of the vaccine under application is significantly minimised by the documented and recorded sourcing of animals (animal-derived material of known and controlled origin), by the nature of animal tissues used in manufacturing (low or no detectable infectivity), by the production processes, and by the negligible risk posed by a series factors which would likely lower the risk if any, such as high dilution of the materials used, route of administration and maximum number of dosage injected. A risk assessment or certification of suitability or declaration of conformity were provided as appropriate, specifically, for MCS and WCS of BHK-21 cells, for MSV and WSV, bovine calf serum (EDQM certificate); for casein of bovine origin and enzymes of porcine origin contained in Glasgow MEM; trypsin (1:250). Certificate statements exclude the presence of any substance of animal origin in the production of saponin. Gelatine (from pig skin), dextran (skimmed milk powder from bovine milk), a reused in the manufacture of microcarriers, and gentamicin sulphate (peptone of fish origin is used in the fermentation process of raw material) are all of no direct rumin origin and thus the risk they pose is negligible. Moreover valid justifications for the use of materials sourced from GBR III level countries were provided.

The documentation supplied all required information. Seed materials and other starting material of animal origin relevant for the transmission of TSE were in compliance with the relevant requirements.

As a result of the above the CVMP concluded that the starting materials of animal origin used in the production of the final product for this vaccine comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

Control tests during production

A detailed description was provided of the in process test controls during production. The general characteristics including validation, where relevant, of these methods were all satisfactorily assessed. They were also assessed satisfactorily during the centralised authorisation procedure of the two monovalent ZULVAC 8 Ovis and ZULVAC 8 Bovis vaccines. The results of the in process control tests carried out on antigen batches produced either in roller bottles and single use bioreactors, were reported and were acceptable.

The list below shows the control tests carried out during the manufacture of BTV-1 and BTV-8 antigens:

Pre-inoculum stage:

- Virus Titration

Inoculum stage:

- Sterility (Ph. Eur.)
- Virus Titration

Final antigen stage:

- Sterility (Ph. Eur.)
- Virus Titration
- Identity

Inactivated and neutralised antigens stage:

- Sterility (Ph. Eur.)
- Inactivation
- Sodium thiosulphate

Bulk vaccine:

- Inactivation (performed on the bulk antigens)
- Absence of pestiviruses (performed on the bulk antigens)
- Absence of Aujeszky's Disease virus (performed on the bulk antigens)
- Sterility (Ph. Eur.) (performed at the end of blending process)

Control tests on the finished product

A detailed description was provided of the in process test controls on the finished product. The general characteristics, including validation, where relevant, of these methods were all satisfactorily assessed. They were also assessed satisfactorily during the authorisation process of two the monovalent ZULVAC 8 Ovis/Bovis vaccines. Relevant data were provided in order to demonstrate that robust and consistent batches of the vaccine under application will be produced.

The list below shows the control tests carried out on the finished product:

- Appearance
- Volume
- Identity: Protection of vaccinated mice against challenge with BTV-1 and BTV-8
- *In vivo* potency: Relative potency by a mice potency test compared to a reference vaccine that was shown efficacious in lambs.
    - BTV-1: RP ≥ 1[*]
    - BTV-8: RP ≥ 1*
- Identification and quantification of the adjuvant aluminium hydroxide gel (Al^{3+})
- Thiomersal
- Safety: No signs of abnormal local or systemic reactions related to the vaccine
- Sterility

[*] Relative potency by a mice potency test compared to a reference vaccine that was shown efficacious in lambs

- Absence of extraneous BTV
- pH

Stability

No specific studies were carried to support the stability of ZULVAC 1+8 Ovis. Some data were presented from 3 batches of ZULVAC 1+8 Ovis tested for up to 6 months (T6); results were satisfactory. As stated in the applicable Guideline on Minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against Bluetongue (EMEA/CVMP/IWP/220193/2008), a maximum shelf life of 12 months can be granted. Due to the need to provide authorised vaccines against Bluetongue serotypes 1 and 8 as soon as possible and the considerable time required in order to complete all stability studies in line with normal requirements the CVMP exceptionally accepted the current limited data with a view for the applicant to provide the remaining information as soon as available. The manufacturer therefore was requested to provide a full set of data to support the stability of the product for the minimum and maximum doses and has also presented the design and timelines of the studies.

The applicant is planning to demonstrate the 12 months stability (at 2°-8°C) of the inactivated antigens of BTV serotypes 1 and 8 by blending a batch of the vaccine from antigen bulks stored for the maximum proposed shelf-life and subsequently showing compliance with the finished product specifications over the proposed shelf-life. This vaccine batch will follow the stability programme submitted to cover the proposed 24 months shelf life. The potency of the batch will be verified according to a provided SOP. The results for T0 at blending were already provided along with a stability plan. In addition to T0, the batch potency test in mice will be carried out at T12 and T24 months, along with physiochemical parameters which will be tested at T0 and T24 . The 24 months stability of minimum and maximum doses of ZULVAC 1+8 Ovis vaccine should be tested according to a monitoring plan which will be conducted up to 27 month and at more frequent intervals.

Three batches of vaccine that were used to demonstrate batch-to-batch consistency were filled into 10 or 120 dose vials (bracketing presentation of the vaccine) and are now on the finished product real-time stability plan. Results obtained at T0 were given above. Results obtained at T3 (3 months) and T6 (6 months) for the batches were acceptable.

Final data are also awaited in order to assess the efficacy of antimicrobial preservatives in ZULVAC 1+8 Ovis vaccine (preliminary results were provided at 5 months (T5)).

Overall conclusions on quality

At present, with the data and clarification provided by the manufacturer, the quality profile of ZULVAC 1+8 Ovis can be considered sufficient for granting a marketing authorisation under exceptional circumstances, when taking into account the benefit-risk balance for BTV serotypes 1 and 8, and when considering the epidemiological situation in the EU.

In this context given that:

- a batch with minimum antigen content was shown efficacious in sheep,
- the production process allows production of consistent batches, with a reliable batch potency test
- current limited data on stability results can be exceptionally accepted due to the need to provide authorised vaccines against Bluetongue serotypes 1 and 8 and the considerable time required in order to complete all stability studies in line with normal requirements the CVMP with a view for the applicant to provide the remaining information as soon as available,

- the provision of information regarding the following remaining outstanding issues on quality are part of the applicant's specific obligations and relate to: a) In process control tests carried out on at least 3 vaccine antigen batches of different sizes, produced within the range of 250-1000 litres b) Final data supporting the 12 months storage time at 2°-8°C of vaccine antigen(s) c) Final data for testing the efficacy of antimicrobial preservation d) Full set of data, according to the reported timelines, in order to demonstrate the claimed stability of finished product e) A validated test to quantify the saponin content in the finished product is awaited

The CVMP has sufficient guarantees to assume that forthcoming batches will be efficacious on sheep when manufactured and released on the basis of the descriptions and specifications laid down in this application as the forthcoming batches will be at least as good as the one used to show efficacy in the target species.

All these assurance were considered sufficient for granting a marketing authorisation under exceptional circumstances, but not for a full marketing authorisation.

3. Safety and residue assessment

Introduction

ZULVAC 1+8 Ovis is a conventionally produced, liquid and ready-to-use, binary ethylenimine inactivated, and aluminium hydroxide /saponin adjuvanted vaccine against BTV serotypes 1 and 8 infections. ZULVAC 1+8 Ovis is recommended for the active immunisation of sheep for the prevention of viraemia induced by infection by BTV-1 and 8 serotypes. A 2ml dose of the vaccine is recommended to be administered by subcutaneous route. The primary vaccination schedule consists of two injections of the vaccine administered to lambs from 1.5 months of age, the second injection given 3 weeks after the initial one.

The safety of the administration of one dose and of repeated administration of one dose and the safety of the administration of an overdose of ZULVAC 1+8 vaccine was investigated under laboratory conditions, in lambs of minimum age recommended for vaccination. The safe use of the vaccine in pregnant ewes was examined in a study farm under restricted conditions. In this category of the target animal species, the safety of the administration of an overdose of the vaccine was also investigated. According to relevant legislation (which allows the omission of field studies) field studies were not performed.

A. Safety assessment

Laboratory safety tests were carried out using a vaccine batch that although its was not standard, it can be considered representative of commercial batches as it was blended according to the standard methods described in the Quality Part of the submitted dossier. The batch was formulated at maximum antigens' concentration per dose (i.e. a maximum of $1x10^{7.0}$ $TCID_{50}$ of each antigen for a total of $2x10^{7.0}$ $TCID_{50}$ per 2ml/dose). Manufacturer's batch protocols of this vaccine batch were provided. The safe use of the vaccine was demonstrated following the recommended vaccinated schedule by administering the first dose of 2 ml in lambs from 1.5 months of age and the 2nd dose after 3 weeks.

Laboratory tests

Safety of the administration of one dose and of repeated administration of one dose

Healthy lambs which had never been vaccinated nor inoculated with other vaccines or substances were enrolled for this study. The animals were allocated randomly into two treatment groups: vaccinated (V) and controls (C). In the vaccinated group (V), lambs were vaccinated three times 3 weeks apart (D0, D21, D42), with 2 ml of ZULVAC 1+8 Ovis, by subcutaneous route.

In the control group (C), lambs were inoculated subcutaneously at the same time with 2 ml of phosphate-buffered saline solution.

Results:

After the administration of the three repeated doses of 2 ml of the vaccine, no systemic reactions were observed; neither the overall health conditions were impaired. No increases in rectal temperature were observed after the 1st vaccination, whereas after the 2nd vaccination, the lambs presented a transient mean rectal temperature increase of 1.19ºC when compared to the mean temperature of the control group on day 1 after the inoculation. On day 2 after vaccination, rectal temperatures were back to normal values. A maximum temperature of 41.84ºC was observed, with only 4 out of 13 vaccinated lambs showing a transient increase in temperature above 41ºC. After the 3rd vaccination lambs presented a transient mean rectal temperature increase of 0.65 ºC (compared to the mean temperature of the control group) on day 1 after the inoculation. On day 2 after vaccination, rectal temperatures were normalised. A maximum temperature of 41.22ºC was observed in a very small number of lambs with only few showing a transient increase above 41ºC 1 day after vaccination. A transient increase in rectal temperature after repeated vaccination was therefore considered an occasional event.

Local reactions at the injection site appeared in all vaccinated lambs after the first and second vaccinations and in 92% after the third vaccination. The average duration of the local reactions was 30 to 44 days, persisting afterwards as small granules of diameter ≤ 0.5 cm in the 54 - 61% of the lambs.

Local reactions varied from generalised diffuse swellings of the whole injection area lasting from 1 to 9 days that evolved into nodules of gradually decreasing diameter from ≥2 to 0.5 cm of diameter (these local reactions appeared in the 77% of the vaccinated lambs after the 1st and 2nd vaccinations, and in the 33% of the lambs after 3rd vaccination); to nodular swellings of ≥2 cm in diameter that gradually decreased to nodules of smaller diameter, 2 to 0.5 cm (these local reactions appeared in the 23% of the lambs after 1st and 2nd vaccinations, and in the 67% of the lambs after 3rd vaccination).

At the post-mortem examination, tissue reactions at the injection sites were observed in all lambs after the first dose with a maximum volume of 0.81 cm^3 and average volume of 0.34 cm^3 and in 85% and 92% of the lambs after the second and third doses respectively, with a maximum volume of 2.7 cm^3 and average volume of 0.6cm^3. The histopathological study showed that tissue lesions correspond to subcutaneous granulomas.

Conclusions:

The safety of the administration of one dose and of a repeated administration of one dose of ZULVAC 1+8 Ovis vaccine was demonstrated in this study and has been adequately reflected under section 4.6 of the SPC which states the following:

"A transient increase in rectal temperature, not exceeding 1.2°C, may occur during the 24 hours following vaccination. Vaccination may be followed in most animals by a local reaction at the injection site. These reactions take the form in most cases of a general swelling of the injection site (persisting

for not more than 7 days) or of palpable nodules (subcutaneous granuloma, possibly persisting for more than 48 days).”

Safety of one administration of an overdose

The administration of an overdose (4 ml) of a batch of ZULVAC 1+8 Ovis formulated at the highest vaccine antigen(s) concentration per dose was proven to be safe in lambs of minimum age. Healthy lambs which had never been vaccinated nor inoculated with other substances were enrolled for this study. The animals were allocated randomly into two treatment groups: vaccinated (V) and controls (C). In the vaccinated group (V), 13 lambs were inoculated on D0 by the subcutaneous route (in the right axillary area) with a double dose (4 ml) of the vaccine under study. In the control group, seven lambs were inoculated at the same time with 4 ml of phosphate-buffered saline solution.

Results:

No systemic reactions were recorded, nor was the general health condition of vaccinated animals impaired. After the administration of an overdose of the vaccine, 85% of the vaccinated lambs (11 out 13 animals) presented local reactions at the site of injection. The reactions appeared on the 1st day after the administration of vaccine, although it was not until day 9 or 10 post vaccination when reactions were present in all reacting animals. The average duration of local reactions was of 36 days with a variation of between 12 and 48 days.

Local reactions were monitored until day 48 post vaccination, when they had disappeared in most of the reacting animals. In general the observed reactions were small granules (diameter ≤0.5 cm) or oedema at the site of injection, which evolved into nodules of 1 to 4 cm in diameter (in 45% of the lambs). In most cases nodules evolved into a generalised swelling of the zone of injection (in 55% of the lambs) for 2 to 9 days during the most severe phase of the reaction; turning again into a small granules during the resolution phase, until they disappeared in 55% of the lambs at the end of the study. At day 48 post vaccination, 27% of lambs still presented with small granules of diameter ≤0.5 cm and 18% of lambs had a nodule of 1 to 2 cm at the injection site.

Conclusions:

The safety of the administration of an overdose (4 ml) of ZULVAC 1+8 Ovis vaccine was demonstrated in this study and has been adequately reflected under section 4.10 of SPC which states:

"*A transient increase in rectal temperature, not exceeding 0.6°C, may occur during the 24 hours following administration of a two-fold overdose. Administration of a two-fold overdose may be followed in most animals by a local reaction at the injection site. These reactions take the form in most cases of a general swelling of the injection site (persisting for not more than 9 days) or of palpable nodules (subcutaneous granuloma, possibly persisting for more than 63 days).”*

Study of the safety of the administration of an overdose of the ZULVAC 1+8 Ovis vaccine to pregnant ewes.

The objective of the study was to verify the safety of the administration of an overdose of the vaccine ZULVAC1+8 Ovis to crossbred pregnant ewes, originating from Spain and at different phases of gestation. The ewes were vaccinated with ZULVAC® 1+8 Ovis, batch E-20, with a concentration for both BTV serotypes of $1x10^7$ $TCID_{50}$/2 ml dose (Total: $2x10^7$ $TCID_{50}$/2ml).

Ewes at different stage of gestation were selected for the study and appropriately allocated to 3 vaccinated (V1, V2, V3) and 3 control (C1, C2, C3) groups in order to have a consistent number of ewes at different stage of gestation in both groups. From those pregnant ewes some were vaccinated by subcutaneous route with 4 ml of ZULVAC 1+8 Ovis, and some pregnant ewes were inoculated with

4 ml of phosphate-buffered saline solution -placebo (control ewes). The appearance of anaphylactic reactions was evaluated after the administration of vaccine or placebo. In the study, rectal temperatures and local reactions at the site of injection were not recorded, in order to avoid the possible reproductive problems associated with the handling of the animals.

Results:

The vaccine did not induce anaphylactic shock in ewes at the different stages of gestation.

The administration of an overdose of vaccine did not affect the reproductive parameters of ewes as the number of ewes that reached parturition, number of abortions, number of lambs born alive healthy and number of lambs born weak or stillborn were within acceptable range.

Conclusions:

The safety of double dose administration of ZULVAC 1+8 vaccine was demonstrated in ewes at different stages of pregnancy.

Safety study of the administration of an overdose of ZULVAC 1+8 Ovis vaccine in pregnant ewes at second phase of gestation.

In the second study ewes at the second stage of gestation (i.e. at approximately 3-5 months of gestation) were enrolled. At the start of the study, the ewes were distributed into 4 treatment groups. Groups V1 and V2 included ewes that were vaccinated (V1). Ewes in groups C1 and C2 were used as controls (C1). All ewes in V1 and V2 were inoculated at D0 by subcutaneous route with a double dose (4 ml) of the vaccine ZULVAC 1+8 Ovis (batch E-20), while the control ewes (C1, C2) were inoculated by subcutaneous route with 4 ml of phosphate-buffered saline solution.

Results:

The vaccine did not induce general reactions, anaphylactic shock or vomiting in the pregnant ewes under test. The ewes presented a slight and transient mean rectal temperature increase of 0.6ºC (compared to the mean temperature of the control group) on day 1 after the inoculation. After the administration of 4 ml of the vaccine ZULVAC 1+8 Ovis, 83% of the ewes presented local reactions at the site of injection. The observed local reactions varied between nodular swellings of 1 to ≥ 2 cm in diameter in the 20% of the ewes to generalised diffuse swellings of 3 to 10 days of duration of the whole area of injection in the 80% of the ewes. In the 40% of the ewes the reactions persisted after day 63 post vaccination as small nodules of diameter ≤ 0.5 cm.

The administration of an overdose of vaccine did not affect the reproductive parameters of ewes at a late phase of gestation as compared to control ewes.

Conclusions:

The safety of double dose administration of ZULVAC 1+8 vaccine was demonstrated in ewes at late stage of pregnancy.

Results from both studies showed that no systemic reactions were induced and the reproductive parameters of vaccinated ewes were not affected. Indeed, comparable results were obtained for vaccinated and control animals. In the study carried out in ewes at the second phase of gestation, special attention was also paid to the potential increase of rectal temperature and to the occurrence of local reactions at injection site. After vaccination, the ewes presented a transient mean rectal temperature increase of 0.6ºC on day 1 after the inoculation. These data were consistent with those observed in lambs of minimum age. After the administration of 4 ml of the vaccine ZULVAC 1+8 Ovis, 83% of the ewes presented local reactions at the site of injection. The observed local reactions varied between nodular swellings of 1 to ≥ 2cm in diameter in the 20% of the ewes to generalised diffuse

swellings of 3 to 10 days of duration of the whole area of injection in the 80% of the ewes. In the 40% of the ewes the reactions persisted after day 63 p.v. as small nodules of diameter ≤0.5 cm.

The safety of the vaccine was not investigated in breeding males. This is reflected under section 4.7 of SPC.

The CVMP considered that according to the results obtained from the two safety studies conducted in pregnant animals, the safety of ZULVAC 1+8 Ovis was adequatedly demonstrated and reflected under sections 4.6, 4.7 and 4.10 of SPC.

Examination of immunological functions

Studies on immunological functions have not been conducted since the vaccine is not expected to affect negatively the immune response of the vaccinated animal, as it is an inactivated vaccine for which the adjuvant has been shown to be safe. This approach was acceptable.

Interactions

As no specific studies were carried out, a recommendation for not mixing with other immunological veterinary medicinal products has been included in the SPC.

Field studies

Data from field studies were not provided. This is in line with the current requirements included in the CVMP Guideline on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/IWP/220193/2008) where is stated that field studies may be omitted. The applicant could not reasonably be expected to provide the results from such trials on the target species due to the difficulties in conducting large scale trials for a disease that is under community control and the need for any experimental studies to be conducted within high containment facilities.

User safety

The applicant provided a detailed risk assessment concerning the potential risk to humans in direct contact with the product and specifically its components, such as the active ingredients, the adjuvant system, residues of antibiotics and preservatives. It was concluded that the nature and concentration level of the constituents, in particular residues of gentamicin and thiomersal, are not susceptible to cause any hazard to the user. The risk of human exposure is limited to the person injecting the product to the animals (veterinarians, or experienced persons working under the direct supervision of a veterinarian). However the amount and method of administration does not pose any additional risks compared to other injectable products to animals and humans. In the absence of irritating substances such as oil adjuvants, the accidental injection of the vaccine into subcutaneous or muscular tissue does not present any specific lesion risks when accidentally injected.

Environmental risk assessment

A Phase I assessment was carried out, providing evidence that there would be no potential risk for the global environment. No phase II assessment was deemed necessary. No hazard should be posed to the environment in light of the nature (inactivated vaccine), and composition of the vaccine under study and the robust manufacturing process. The manufactirong process includes a fully validated inactivation test and control of inactivation and thus there is no risk arising from the presence of live Bluetongue virus in the vaccine and its excretion into the environment. The excipients consist of

substances that will likewise not be excreted into the environment. The vaccine is contained in high density polyethylene bottles with rubber stoppers. The probability that the accidental opening of a vial may become a risk to the environment is insignificant. In the event that the vaccine reaches the environment, such as in the case of vial breakage or spilling of vaccine, the amounts will be very small and, therefore, it is not expected that such situation will have any measurable effects on the environment. Based on these considerations, the general risk posed to the environment by the use of the vaccine under study is negligible.

B. Residue Assessment

Study of residues

The applicant has provided supportive evidence based on the well known qualitative characteristics of the vaccine components and on the minimum amount of vaccine administered to the animals for the absence of any specific study of residues being conducted.

Statement of the MRLs

The active substance being a principle of biological origin intended to produce active immunity is not in the scope of Regulation (EC) No 470/2009.

The excipients, including adjuvants, listed in section 6.1 of the SPC are either allowed substances for which Table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Overall conclusion on safety

Sufficient data were provided in order to specifically assess the potential risks arising from the use of ZULVAC 1-8 Ovis in sheep. Laboratory studies with the vaccine under application were provided.

Under the tested conditions, the vaccine was generally well tolerated as demonstrated by the absence of major systemic reactions impacting body temperature and growth performances following administration in sheep. Local reactions were acceptable in terms of size, frequency of occurrence, and duration. Studies regarding the reproductive performances were provided that were conducted on pregnant ewes; no impact on the offspring was reported. The safe use of the vaccine in breeding males was not investigated and references to that have been noted in the relevant section of the SPC. Data from field trials were not provided, in line with current requirements. No specific residue studies were carried out but a withdrawal period of zero days has been justified. The consequence and level of risk arising from its use are negligible as conclusion from the phase I of the environmental risk assessment.

In relation to the safety in lactating sheep, the relevant section of the SPC and labelling was revised to reflect the current lack of data.

Evidence was provided that showed that there is no potential risk for the environment. For the user there is a very low risk of self injection. However in the absence of irritating substances such as oil adjuvants, the accidental injection of the vaccine into subcutaneous or muscular tissue does not present any specific risks when accidentally injected.

4. Efficacy assessment

Introduction

The vaccine is recommended for the active immunisation of sheep in order to prevent viraemia established in animals infected by serotype 1 and 8 of BTV. A 2 ml dose of the vaccine is recommended to be administered by subcutaneous route to sheep (including pregnant animals). The basic vaccination schedule consists of one initial injection given from a minimum of 1.5 month of age and followed by a second injection given 3 weeks later. Onset of immunity is 3 weeks after the completion of the basic vaccination course. The duration of immunity (DoI) is 1 year. The absence of any investigation of the influence of maternally derived antibodies (MDA) on the vaccine's efficacy is reflected in a statement included in SPC. Field trials were not strictly required for this type of application. A DIVA strategy has not been implemented yet.

A summary of the vaccine batches used in laboratory efficacy studies was provided and they were considered appropriate for the use in efficacy studies.

Since it is expected that BTV vaccines are capable to prevent viraemia, the following definition of prevention was agreed to substantiate the claim for protection against BTV viraemia: consistent absence of viral load detectable by real time qRT-PCR (segment 5, Toussaint et al, 2007) in all the vaccinated animals during the monitoring period of minimum 4 weeks. Viral load detectable by real time qRT-PCR was defined as the one that provides a result of Ct[†] value lower than 36.0 in vaccinated animals (thus predicting the interruption of virus transmission). During the qRT-PCR validation studies, its sensitivity in virus suspension was 2 $TCID_{50}$/ml for BTV-1 and 1.8 $TCID_{50}$/ml for BTV-8.

The main goal for establishing a suitable challenge model was to inoculate an amount of virus so that all control animals become viraemic during the study (from approximately 5 days to 27 days post infection). Challenge models were established for both BTV-1 and BTV-8. A validated qRT-PCR was used for detection of BTV genome in experimentally challenged animals.

Challenge Model for BTV-1

The challenge strain used in the efficacy studies was homologous to the vaccine strain. This was not considered an ideal condition in order to assess the efficacy of the vaccine; however the emergency situation justified the use of the only available homologous virus strain. The strain (which originates from Algeria) was isolated in September 2006 by the Institute of Animal Health, Pirbright, UK. The challenge dose was established by the Spanish Reference Laboratory for BTV (i.e. Laboratorio Central de Veterinaria-(LCV)). The challenge inoculum consisted of a predetermined challenge virus suspension, administered to lambs by subcutaneous route. Supportive evidence was provided that since the identity of the European isolates that were identified as BTV-1 which also determines the specificity of neutralisation and virus serotype, the vaccine strain of BTV-1 from Algeria should cross-protect fully against all of the later European BTV-1 isolates, even if they are reassortants.

Challenge Model for BTV-8

The challenge strain used in the efficacy studies was homologous to the vaccine strain. This was not considered an ideal condition in order to assess the efficacy of the vaccine; however the emergency situation somehow justified the use of the only available homologous virus strain. This virus strain was isolated in Belgium from the blood of a sheep that was clinically affected by Bluetongue virus. The challenge dose was established by the LCV, reference laboratory in Spain for BTV. The challenge

[†] Threshold cycle number in PCR assay

inoculum consisted of a predetermined challenge virus suspension administered to lambs by subcutaneous route.

For both BTV serotypes, the challenge model included a 4 week period of observation of the challenged animals. Animals were monitored for the appearance of major clinical signs of BTV-1 or BTV-8 infection, including increase of rectal temperature, nasal/ocular discharge, nasal/ocular oedema and lameness. Blood samples for assessing the presence of viraemia (by the validated qRT-PCR) in challenged animals were periodically collected until 4 weeks after challenge.

Several points for concern and clarification were addressed to the applicant, notably with reference to the use of homologous challenge to assess the efficacy of both BTV components of ZULVAC 1+8 Ovis vaccine, the suitability of the challenge virus strain with regard to the current epidemiological situation in EU Countries, to the clinical outcome of the experimental challenge, to the absence of any comparative evaluation of the results obtained by the validated qRT-PCR and virus isolation on eggs or cell culture.

Laboratory trials

Determination of the vaccine dose and onset of immunity

Pre-immunogenicity study of ZULVAC 1+8 Ovis vaccine in 1.5-month-old lambs

The objective of this study was to evaluate the efficacy of four different antigen concentrations of the ZULVAC 1+8 Ovis vaccine, in order to establish the lowest concentration tested which is able to prevent viraemia (the presence of viral genome in the blood by real time RT-PCR in vaccinated lambs compared to unvaccinated animals) in 100% of vaccinated lambs.

The lambs were randomly allocated into 5 treatment groups of animals: G1-2-3-4, each consisted of lambs vaccinated at Day 0 and revaccinated at D20 by subcutaneous route, with a 2 ml dose of the corresponding vaccine preparation; G5, consisted of control lambs which did not receive any type of placebo treatment. Blood sampling was carried out at established time points in order to monitor the serological response (sero-neutralising (SN) antibodies) after vaccination. Twenty-one days after second vaccination (D41), half of the animals of each vaccinated group and 75% of the control group were submitted to a virulent challenge with BTV-1 and BTV-8 by subcutaneous route. Blood samples were taken from all animals after challenge.

Results:

Uptake of the vaccine was demonstrated 2 weeks after completion of the basic vaccination scheme. The SN titres to BTV-8 declined until challenge. Control lambs remained negative at all bleeding time points. BTV-1 and BTV-8 genome was not detected in any of vaccinated lambs at any time points during 27 days after challenge, whereas in all the non-vaccinated and challenged lambs, BTV-1 and BTV-8 genome was detected starting from D4 post infection. No statistically significant differences regarding rectal temperatures after BTV-1 and BTV-8 challenge were recorded between the vaccinated groups, whereas there were statistically significant differences regarding rectal temperatures after BTV-1 and BTV-8 challenge between the vaccinated groups and the control group on days: 4, 6, 8, 12, and 22 after BTV-1 challenge and on days 6 and 8 after BTV-8 challenge. Other clinical signs attributed to BTV-1 and BTV-8 infection, such as nasal discharge and/or oedema, ocular discharge and/or ocular oedema, lameness and prostration, were recorded in some groups of vaccinated lambs as well as in control lambs during the observation period (other clinical signs not associated to BTV infection were also recorded).

Conclusions:

The different antigen concentrations in ZULVAC 1+8 Ovis vaccine batches tested in this study induced an active immunity for BTV-1 and BTV-8 serotypes able to prevent viraemia in the vaccinated and challenged lambs during 4 weeks after challenge. The minimum content of $10^{6.1}TCID_{50}$/dose provided full prevention of viraemia. Onset of immunity was set at 3 weeks after completion of the basic vaccination scheme.

Efficacy study of the administration of 2-shots ZULVAC 1+8 Ovis vaccine in 1.5-month-old lambs

The objective of this study was to evaluate the efficacy of two different antigen concentrations of the ZULVAC 1+8 Ovis vaccine, in order to test if they were able prevent viraemia in 100% of vaccinated animals.

The lambs were randomly allocated into 3 treatment groups of animals. Lambs in G1 and G2 were vaccinated at D0 and revaccinated at D22 by subcutaneous route, with a 2 ml dose of the corresponding vaccine preparation. Lambs of G3 were left as unvaccinated controls, and did not receive any type of placebo treatment. After vaccination/re-vaccination, the animals were monitored for the potential occurrence of any systemic reactions associated with the administration of the vaccine. Blood sampling was carried out at established time points in order to monitor the serological response after vaccination. Three weeks after vaccination (D43), half of the animals of each vaccinated group were submitted to a virulent challenge by subcutaneous route with a virulent suspension of BTV-1 and BTV-8. Blood samples were taken from all animals after challenge (clinical signs were also recorded) for the evaluation of the presence of BTV genome by the validated qRT-PCR.

Results:

The evolution of seroneutralising antibodies against BTV-1 and BTV-8 from vaccination on D36 (2 weeks after the 2nd vaccination) and until D43, at challenge was provided. The uptake of the vaccine was demonstrated 2 weeks after completion of the basic vaccination scheme. Control lambs remained negative at all bleeding time points. The BTV genome was not detected in any of vaccinated lambs at any time points during the 27 days after challenge, whereas in all the non vaccinated challenged lambs, BTV genome was detected starting from D3 and D6 post infection, in animals challenged with BTV-1 and BTV-8, respectively. After BTV-1 and BTV-8 challenge, there were no significant statistical differences regarding rectal temperatures between the vaccinated groups. Significant statistical differences were found regarding rectal temperatures between the vaccinated groups (considered as one group) and the control group respectively on days: 3, 6, 8 and 10 after BTV-1 and BTV-8 challenge.

Conclusions:

In vaccinated lambs, the two different antigen(s) concentrations in ZULVAC 1+8 Ovis vaccine induced an active immunity (for both serotypes) able to prevent viraemia after the challenge carried out 3 weeks after completion of the basic vaccination scheme. Based on the results obtained from this study, the batch used containing $10^{6.5}$ $TCID_{50}$ /2ml dose was designated as the reference vaccine for the potency test (RP=1). After BTV-1 challenge, vaccination reduced hyperthermia and clinical signs. After BTV-8 challenge, no reduction of hyperthermia and clinical signs were demonstrated in the vaccinated lambs, since less and milder clinical signs were registered in the control lambs.

Conclusions concerning minimum efficacy dose and onset of immunity

The results obtained from both studies described above demonstrated that ZULVAC 1+8 Ovis with a minimum antigen content of $10^{6.1}$ $TCID_{50}$/ml prevents viraemia following the completion of the two

doses vaccination scheme, from 6 weeks of age, with an onset of immunity of 21 days following the second vaccination. Batch E-22 (used in the second study above) containing $10^{6.5}$ $TCID_{50}$ /dose based on pre-inactivation titre, has been designated as the reference vaccine for the potency test (RP=1). Therefore for batch release all vaccines must show a minimum relative potency to this vaccine of ≥1, and minimum antigen content for blending is set at a pre-inactivation titre of $10^{6.5}$ TCID50/dose for each vaccine antigen.

As a result the vaccine can be indicated for active immunisation of sheep from 1.5 months of age for the prevention of viraemia caused by Bluetongue Virus, serotypes 1 and 8.

Influence of maternal antibody on the efficacy of the vaccine.

The efficacy of the vaccine in the presence of MDAs was not investigated. A warning it has been included in the relevant section of SPC.

Determination of the duration of immunity (DoI)

Results were provided from two studies conducted with monovalent ZULVAC 1 Ovis and ZULVAC 8 Ovis that provided some supportive evidence that the bivalent vaccine should be able to induce full protection for up to 1 year. Furthermore, the final report of the ZULVAC 1+8 Ovis Duration of Immunity Study was provided together with a Response to Booster Study.

The data provided showed full prevention of viraemia upon challenge 12 months after the primary vaccination course, consistent with the SPC claim section 4.2 for both BTV-1 and BTV-8. In addition the response to booster study demonstrated an anamnestic response and full protection from challenge 3 weeks following the booster (1 dose) vaccination for both BTV-1 and BTV-8. However, the duration of immunity after this single (1 dose) booster has not been proven.

Duration of immunity study of ZULVAC 1+8 Ovis 2-shots vaccine in lambs

The objective of the study was to evaluate the ability of ZULVAC 1+8 Ovis vaccine to prevent viraemia (no detection of viral genome by qRT-PCR technique during 27 days post challenge) in sheep challenged 12 months after completion of the primary vaccination scheme. Two of ZULVAC 1+8 Ovis vaccine were used, which were formulated at a concentration of $10^{6.7}$ and $10^{6.5}$TCID50/2ml dose for both antigen serotypes for each batch respectively. The BTV-8 content of the ZULVAC 1+8 Ovis is 0.1 log was higher than for the monovalent ZULVAC 8 Ovis i.e. 6.5 vs 6.4 log10 TCID50, however, such a difference was not considered of major impact on the assessment as it is within the variability of the titration test method and the efficacy of both products was fully confirmed. The Manufacturer's Batch Protocols (MBPs) were provided for the two batches of ZULVAC 1+8 vaccine used.

Healthy 8-9 weeks old, crossbred lambs, without antibodies against BTV, were included in the study. Almost half of those lambs were used for the 12 months DoI challenge study, whereas the remaining animals were kept for the anamnestic response study. The lambs were allocated to 3 treatment groups (1-2-3). In groups 1 and 2, lambs were vaccinated with batches containing $10^{6.7}$ and $10^{6.5}$TCID50/2ml dose for both antigen serotypes, respectively, according to the recommended scheme of vaccination and route of administration (one vaccination followed by a second dose given 3 weeks later, was administered by subcutaneous route on Day 0 (D0) and Day 21 (D+21), respectively). Lambs in group 3 were left as unvaccinated controls. After vaccination, sheep were monitored for the appearance of any systemic reaction associated with the vaccine administration (anaphylactic shock, anorexia, etc.).

Twelve months after completion of the primary vaccination scheme the vaccinated sheep were submitted to an experimental challenge given by subcutaneous route using a virus suspension of BTV-1and BTV-8 respectively. In both cases the challenge virus strain was homologous to the vaccine strain. This

condition was not considered ideal for such type of experiment, however both challenge virus strains were considered relevant to the epidemiologic situation in Europe, therefore acceptable in order to demonstrate the efficacy of the two batches of ZULVAC 1+8 vaccine. The efficacy of the vaccine batches was assessed based on the definition of protection: consistent absence of viral load detectable by qRT-PCR (segment 7, according to Toussaint et al, 2007) in all vaccinated animals during the monitoring period of 4 weeks. The defining viral load detectable by qRT-PCR was a Ct value <36.0.

One year after completion of the basic 2 shots vaccination scheme, some lambs from each group, were included in an anamnestic response study (lambs in group 1 and 2 received a booster vaccination, whereas lambs in group 3 were still left as unvaccinated controls. Three weeks later, half of the lambs from each group, were submitted to a virulent challenge with BTV-1 or BTV 8) for the evaluation of viraemia after challenge.

The monitored clinical signs were rectal temperature increase; lameness; prostration; death. In order to obtain the daily clinical score, a value of 1 was attributed to each clinical sign that the lambs presented, except for death when value of 3 was attributed. At the end of the study, i.e. after 27 days after challenge, all lambs were euthanized.

Bleeding was also carried out on D 0 before the 1st vaccination, and frequently thereafter.

Results:

The vaccine was well tolerated by all lambs which never manifested systemic reactions such as anaphylactic shock, anorexia, prostration, after the 1st and 2nd vaccination. At D0, none of the lambs selected for the study presented antibodies against any of BTV serotypes by ELISA. Also, at D0, in none of the lambs viral genome was detected by qRT-PCR. The evolution of the geometric mean titres (GMTs) of serum neutralising antibodies against BTV-1 and BTV-8 from vaccination until challenge was presented.

Statistically significant differences were recorded, concerning the increase of rectal temperatures between vaccinated and control groups on D+5, 7 and 10 after challenge coinciding with the period of maximal viraemia recorded in the unvaccinated sheep. The clinical outcome of both BTV-1 and BTV-8 challenge was of very limited extent. Vaccinated sheep in group 1 did not manifest any clinical sign attributable to BTV infection at any time point during the monitoring period after challenge. Most animals in group 2 did not manifest, at any time after challenge, any clinical sign attributable to BTV infection. A small number of control sheep (from each BTV serotype) died after challenge. Evidence was provided that the death of some controls was due to BTV-1 and BTV-8 infection. The remaining control lambs presented very mild, unspecific clinical signs of BTV infection after challenge. No statistical significant differences were recorded among groups. In none of the vaccinated sheep of both groups 1 and 2 challenged with both BTV-1 and BTV-8, viral genome detected by qRT-PCR at any time point checked during 27 days after challenge. Contrary, in all the unvaccinated sheep the viral genome was detected from D+3 after challenge with BTV-1 and from D+5 after challenge with BTV-8 up to 27 days after challenge when the study terminated.

Conclusions:

The efficacy of ZULVAC 1+8 Ovis vaccine in terms of prevention of viraemia in vaccinated and challenged sheep for 12 months was supported by the results of the study.

Anamnestic response study of ZULVAC 1+8 Ovis in sheep

The objective of this study was to test the anamnestic response in sheep, after the administration of a booster vaccination of ZULVAC 1+8 Ovis vaccine given to sheep 12 months after completion of primary vaccination scheme. The anamnestic response was measured in terms of capability of the vaccine to

prevent the viraemia (detection of viral genome by qRT-PCR) caused by a homologous BTV-1 and BTV-8 experimental challenge.

Two batches of the ZULVAC 1+8 vaccine were used for this study formulated, respectively, at a concentration of $10^{6.7}$ and $10^{6.5}$TCID50/2ml dose of both antigen serotypes for each batch. The BTV-8 content of the ZULVAC 1+8 Ovis is 0.1 log higher than for the monovalent ZULVAC 8 Ovis i.e. 6.5 vs 6.4 log10 TCID50, however, such a difference was not considered significant as being within the variability of the titration test method and the efficacy of both products has been fully confirmed. The Manufacturer's Batch Protocols were provided, and the composition of one dose was detailed.

Healthy sheep from the study on duration of immunity in lambs with ZULVAC 1+8 Ovis 2-shots described above, were used for this anamnestic response study. Specifically, some from group 1, some from group 2 and some from group 3.

The sheep received a different treatment depending on the groups they were allocated in the duration of immunity study (115-O1-E-19-09) and specifically:

Group 1: Sheep vaccinated according to the primary vaccination scheme 1 year before with a batch containing $10^{6.7}$ TCID50/2ml dose of ZULVAC 1+8 Ovis, were vaccinated (one 2ml/dose) by subcutaneous route with another batch also containing $10^{6.7}$ aTCID50/2ml dose of ZULVAC 1+8 Ovis.

Group 2: Sheep vaccinated according to the primary vaccination scheme 1 year before with a batch containing $10^{6.5}$ TCID50/2ml dose ZULVAC1+8 Ovis, were vaccinated (one 2ml/dose) by subcutaneous route with another batch containing $10^{6.5}$TCID50/2ml dose ZULVAC 1+8 Ovis vaccine.

Group 3: control sheep were left as unvaccinated controls.

After vaccination, sheep were monitored for the appearance of systemic reactions associated with the vaccine administration (anaphylactic shock, anorexia, etc.). Blood samples were taken from all the sheep at Day 0 (before booster vaccination) and 21 days later at challenge (D+21), in order to measure the serum neutralising antibody titres in the animals selected for this study.

On day D+21, 5 sheep of each group (1, 2, and 3) were challenged with BTV-1 and other 5 sheep of each group (1, 2 and 3) were challenged with BTV-8.

In both cases, the challenge strain was homologous to the vaccine strain. This condition was not considered ideal for this type of experiment, however both challenge virus strains were considered relevant to the epidemiologic situation in Europe and therefore acceptable in order to demonstrate the efficacy of the two batches of ZULVAC 1+8 vaccine. The efficacy of the vaccine batches was assessed based on the definition of protection as consistent absence of viral load detectable by qRT-PCR (segment 7 according to Toussaint et al, 2007) in all vaccinated animals during the monitoring period of 4 weeks. The viral load which was detectable by qRT-PCR was defined as the one that provides, a result of a Ct value <36.0.

Blood samples were taken from all the animals just before challenge (D0 post infection), and frequently thereafter, for the evaluation of the presence of the BTV genome by qRT-PCR.

The animals were monitored on days 0, 4, 6, 8, 11, 14, 18, 21, 25 and 28 after challenge for the appearance of clinical signs associated with the disease.

Results:

None of the sheep manifested any systemic reactions (anaphylactic shocks, anorexia, prostration) after vaccination.

At Day 0, all the sheep from vaccinated groups presented antibodies against BTV serotype 1 and 8 whereas none of the control sheep had antibodies. The booster (D+21) vaccination on D+21 resulted in an increase of neutralising antibody titres against BTV-1 and BTV-8 in all the vaccinated sheep. In none of the sheep, BTV genome was detected on the day of challenge.

The evolution (geometric mean titres-GMTs) of neutralising antibody titres against BTV-1 and BTV-8 in sheep of groups 1, 2 and 3, from vaccination to challenge is presented below:

GROUP	GMTs of neutralizing antibodies against BTV-1 and BTV-8			
	D0		D+21	
	BTV-1	BTV-8	BTV-1	BTV-8
1	45.9	19.0	724.1	175.9
2	44.6	16.4	362.0	128.0
3	<2	<2	<2	<2

In none of the sheep from group 1 and group 2 challenged with BTV serotype 1 and BTV serotype 8, viral genome was detected by real time RT-PCR during 28 days after challenge. In all the unvaccinated and challenged sheep of group 3, the viral genome was detected from D+4 after challenge with BTV-1 and BTV-8.

With regard to BTV-1 serotype a statistically significant difference was recorded in relation to the increase of rectal temperatures between vaccinated and control groups on D+8 after challenge. Similarly a significant statistical difference was recorded with regard to BTV-8 serotype, in relation to the increase of rectal temperatures between vaccinated and control groups on D+6 and D+8 after challenge. In both cases the controls had higher values.

Clinical signs attributable to BTV infection were practically absent during the monitoring period after challenge although a control was euthanised due to severe infection indicative of BTV.

Conclusions:

The results obtained from this study demonstrated that the administration of a booster vaccination of ZULVAC 1+8 Ovis one year after a primary vaccination course induced an anamnestic response in the sheep able to prevent viraemia in the vaccinated sheep challenged 21 days (coinciding with the established onset of immunity) after the booster vaccination with BTV serotypes 1 and 8. However any further duration of the booster effect cannot be extrapolated.

Additional studies

No additional studies were reported (e.g. efficacy in other non-target ruminant species).

Field trials

Data on field trials were not provided. In light of the current requirements in the CVMP Guideline on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/ /IWP/220193/2008), field trials may be omitted. The applicant could not reasonably be expected to provide the results from such trials on the target species due to the difficulties in conducting large scale trials for a disease that is under community control and the need for any experimental studies to be conducted within high containment facilities.

Overall conclusion on efficacy

Data of four laboratory studies were provided in order to support the efficacy of the ZULVAC 1-8 vaccine; these were challenge studies where animals were challenged with both BTV-1 and BTV-8 serotypes using vaccines containing low antigen payloads for both serotypes in lambs of young age. The proposed indication for use in sheep as reflected in the SPC is considered as being supported by these studies.

The applicant provided a study with the vaccine under application to support duration of immunity for 12 months. The anamnestic response of a booster vaccination one year after the primary vaccination course was studied in sheep and it was able to prevent viraemia in the vaccinated sheep challenged 21 days after the booster vaccination with BTV serotypes 1 and 8. However the duration of the booster vaccination was not investigated. Field trials were not provided; this was also exceptionally accepted as the applicant could not reasonably be expected to provide the results from such trials on the target species due to the difficulties in conducting large scale trials for a disease that is under community control and the need for any experimental studies to be conducted within high containment facilities.

The efficacy of the proposed vaccination scheme in breeding males and the impact of the acquired maternal immunity on the efficacy when vaccinating young animals, were not investigated. Therefore specific warnings in the relevant sections of the SPC were included. The efficacy in pregnant animals was also not investigated, but the safety was assessed and demonstrated. All these circumstances have been reflected in the SPC.

Neither DIVA strategy was implemented, nor data were provided in relation to the development of any strategy allowing the differentiation between infected and vaccinated animals.

Within the context of an authorisation given under exceptional circumstances, and consistently with the provisions in the relevant guideline and the inclusion of specific warnings in the relevant sections of the SPC, the efficacy of the product can be considered as acceptable.

5. Benefit risk assessment

Benefit assessment

Direct benefit

ZULVAC 1+8 Ovis is an inactivated vaccine conventionally produced that induces an active immunisation of sheep from 1.5 months of age and has been shown to prevent viraemia established in animals infected by serotype 1 and 8 of BTV. The onset of immunity is 3 weeks after the completion of the basic vaccination course and the duration of immunity of 12 months has been adequately supported. In view of the epidemiological situation, the lack of authorised products (in particular bivalent with the combination of serotypes 1 and 8) and the potential for epizootic spread if urgent measures, including vaccination, are not taken to control the disease at EU level, this application is being considered for an authorisation under exceptional circumstances.

Direct therapeutic benefits

Vaccines are a well established and effective method to control the spread of bluetongue virus.

The objective is to induce sufficient immunity to reduce the level of viraemia below a level where transmission could occur and decrease the impact of clinical signs.

Clinical trials demonstrated that the product is capable of inducing an immune response which prevents viraemia and reduces clinical signs in sheep and last for 12 months after primary vaccination. The effect is to prevent transmission and minimise the impact of clinical signs.

Additional benefits

ZULVAC 1+8 Ovis is a standard inactivated vaccine and as such fits in with accepted vaccination practice in the field.

Vaccination has been shown to be safe for use during pregnancy in sheep, which is valuable during a widespread vaccination programme usually necessary to control the spread of disease.

The vaccine is inactivated by a validated inactivation method therefore there are no risks of spread of live virus.

The vaccine is a bivalent vaccine thus enabling protection against 2 serotypes at the same time while administering one product and following one vaccination schedule.

Risk assessment

Main potential risks

a) There is a risk of a transient increase in rectal temperature, not exceeding 1.2°C, which may occur during the 24 hours following vaccination. Vaccination may be followed in most animals by a local reaction at the injection site. These reactions take the form in most cases of a general swelling of the injection site (persisting for not more than 7 days) or of palpable nodules (subcutaneous granuloma, possibly persisting for more than 48 days).

b) For the user there is a low risk of self injection. However in the absence of irritating substances such as oil adjuvants, the accidental injection of the vaccine into subcutaneous or muscular tissue does not present any specific risks when accidentally injected.

c) For the environment there is negligible risk that the vaccine components may cause unexpected effects to the environment.

d) For the consumer there are no components which require an MRL, therefore there are no concerns over failure to observe an MRL. The product contains components found in other marketed products and therefore the risk is no greater than already exists.

Specific potential risks, according to product type and application

Limited data are available on the stability of product during storage. It is permissible for a preliminary shelf life of 12 months to be granted for this product due to its exceptional nature. Nevertheless there is a risk that the product may not be stable for this period. Due to the need to provide authorised vaccines against Bluetongue serotype 1 and 8 as soon as possible and the considerable time required in order to complete all stability studies in line with normal requirements the CVMP can exceptionally accept the current limited data with a view for the applicant to provide the remaining information as soon as available.

Risk management or mitigation measures

a) Appropriate warnings have been placed in the SPC to warn of the potential risks to the target animal and environment.

b) Additionally, no special concern is posed by the final product in light of the safety of packaging, of the number of injections and of the maximum quantity administered to animals, of the route and of the method of administration, and disposal.

Evaluation of the benefit risk balance

The product has been shown to have a positive benefit risk balance for use in sheep. The product has been shown to be efficacious for the indication of viraemia prevention and reduction of clinical signs. The duration of immunity was demonstrated to be 12 months.

The formulation and manufacture of ZULVAC 1+8 Ovis are largely well described and specifications are supported. The applicant is able to detect sub-potent batches thereby ensuring that product of consistent quality will be produced.

The product is well tolerated by the target animals and presents a low risk for users and the environment and appropriate warnings have been included in the SPC. The withdrawal period is zero days.

Conclusion on benefit risk balance

The information provided in the dossier and in response to points raised is sufficient to confirm an overall positive benefit risk balance under exceptional circumstances. The reasons which were considered as relevant in order to acknowledge the exceptional circumstances status of this application were the following:

- Bluetongue disease is spread by insect vectors and therefore presents particular challenges in terms of control due to an inability to prevent transmission from infected animals other than through insect control combined with reducing or preventing viraemia (virus in the blood) in susceptible animals by means of vaccination
- Bluetongue disease is epizootic in nature and has the potential to result in high morbidity and mortality in susceptible populations, particularly of sheep
- Over the last ten years the Bluetongue epidemiological situation in Europe has changed considerably, with the incursion of new serotypes that have been never reported before and with outbreaks in areas which until now were not considered at risk of bluetongue, as is the case for BTV 8 serotype. Also with the outbreaks of BTV-1 declared in Spain, Portugal and France and the possibility of spread of this serotype to other regions and countries in Europe is notice and the possible co-infection with both serotypes has also occurred, with not well studied consequences of the epidemiology and pathology of the disease.
- There are still a small number of vaccines against bluetongue in Europe authorised via the centralised procedure.
- That consequently any delay should be avoided where possible in making available safe and effective vaccines that have been demonstrated to be in compliance with the CVMP guideline on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/IWP/220193/2008).

Moreover, the following were acknowledged in relation to remaining outstanding information:

- The applicant cannot reasonably be expected to provide the results from certain trials on the target species due to the difficulties in conducting large scale trials for a disease that is under community control and the need for any experimental studies to be conducted within high containment facilities.

- Considerable time is required in order to complete all stability studies in line with normal requirements and therefore the CVMP could exceptionally accept the current limited data with a view for the applicant to provide the remaining information as soon as available.
- The applicant should provide information on remaining quality outstanding issues as they form part of the applicant's specific obligations and relate to: a) in process control tests carried out on at least 3 vaccine antigen batches of different sizes, produced within the range of 250-1000 litres, b) final data supporting the 12 months storage time at 2°-8°C of vaccine antigen(s) c) final data for testing the efficacy of antimicrobial preservation, d) full set of data, according to the reported timelines, in order to demonstrate the claimed stability of finished product, e) a validated test to quantify the saponin content in the finished product is awaited.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the overall benefit-risk balance was considered favourable for authorisation under exceptional circumstances.

In addition, data on the stability of the vaccine, results from in-process control tests carried out on at least 3 vaccine antigen batches of different sizes, produced within the range of 250-1000 litres and a validated test to quantify the saponin content should be provided as stated in the specific obligations of the opinion. Satisfactory answers must be given also to all points that require resolution in order for the authorisation to revert to normal status.