SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

Zulvac 8 Bovis, is a conventionally produced, liquid and ready-to-use, BEI inactivated vaccine, adjuvanted with aluminium hydroxide ($Al(OH)_3$) and saponin. The vaccine is intended for the active immunisation of cattle from 3 months of age for the prevention of viraemia caused by bluetongue virus (BTV), serotype 8. The active substance of Zulvac 8 Bovis is the inactivated bluetongue virus serotype 8.

The benefit of Zulvac 8 Bovis is that it induces an active immunity in cattle against bluetongue virus, serotype 8. The vaccine dose is 2 ml. The basic vaccination schedule consists of one injection given intramuscularly from a minimum of 3 months of age followed by a second injection given 3 weeks later. Onset of immunity is 25 days after the completion of the basic vaccination course. The duration of immunity (DoI) has not been fully established yet but interim result support one of at least 6 months. As a consequence, any revaccination scheme should be agreed by the Competent Authority or by the responsible veterinarian, taking into account the local epidemiological situation. The most common side effect is a potential slight and transient but significant increase in the mean rectal temperature of 0.4ºC in the vaccinated calves after the second injection of a single dose during the first 24 hours.

Although the bluetongue disease is less common in cattle, some clinical signs have appeared in recent epizootics in Northern West Europe caused by the BTV 8 serotype. The most prominent lesions in BTV-8 infected cattle included nasal discharge, crusts/lesions of the nasal mucosa, salivation, fever, conjunctivitis, dysphagia, depression, congestions of the oral mucosa, redness of the skin, swollen teats and lameness.
Over the last ten years, the bluetongue situation in the EU has considerably changed with incursions of new serotypes, particularly of serotype 8 into an area of the Community where outbreaks had not been reported before and which was not considered at risk of bluetongue. Outbreaks due to serotype 8 occurred in the Netherlands, in Belgium, Germany, Luxemburg, France and in the UK. It is considered likely that the disease will remain in Europe for the next few years creating an endemic situation.

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 Reflection Paper on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/ /IWP/105008/2007) which later into the procedure was developed into a guideline (EMEA/CVMP/IWP/220193/2008).

2. QUALITY ASSESSMENT

COMPOSITION

Composition for dose of 2 ml is provided in the following table.

Names of ingredients		Quantity per 2 ml dose	Function	Reference to standards
Active substance	Inactivated Bluetongue virus (BTVi) serotype 8, strain BEL2006/02	RP* ≥ 1	antigen	In-house monograph
Constituents of the adjuvant				
Aluminum hydroxide		4 mg Al^{3+}	Adjuvant	Eur. Ph. monograph 1664
Saponin		0.4 mg	Adjuvant	In-house monograph
Excipient Including Component of saline solution	Thiomersal	0.2 mg	Preservative	Eur. Ph. monograph 1625
	Saline solution	qs 2 ml	Volume adjustment	
	Sodium chloride			Eur.Ph. mn. 193
	Potassium chloride			Eur.Ph. mn. 185
	Disodium phosphate dihydrate			Eur.Ph. mn. 602
	Potassium dihydrogen phosphate			Eur.Ph. mn. 920
	Water for injections			Eur.Ph. mn. 169

*Relative Potency by a mice potency test compared to a reference vaccine that was shown efficacious in cattle.

The blending of vaccine is based on the infection titre ($TCID_{50}$/ml) before inactivation. A batch potency test in mice suitable to discriminate between potent and sub-potent batches of the vaccine was also developed by the Applicant.

Containers

The vaccine is filled in 20 ml (corresponding to 10 doses of 2 ml) and 100 ml (corresponding to 50 doses of 2 ml) capacity glass (hydrolytic type I and type I and II respectively for bottles of 20 ml and 100 ml capacity) bottles (complying with Eur. Ph. monograph 3.2.1), closed with a butyl rubber stopper (Eur. Ph 3.2.9) and sealed with an aluminium cap. Tests of compliance with Eur. Ph were provided.

DEVELOPMENT PHARMACEUTICS

A BTV strain was isolated from the blood of an infected sheep during an outbreak of bluetongue in Belgium in 2006 and was used as the active ingredient for the production of Zulvac 8 Bovis. The

production is based on virus and cell seed lot systems. Materials used for the production of both active ingredient and final product as well as relevant manufacturing processes were either classical or conventional ones. The vaccine is filled in multi-dose bottles and for this reason thiomersal is added as preservative. The virus is grown on BHK-21 cells; thereafter, the virus harvest is titrated to determine the number of virus particles per ml capable to infect BHK-21 cells ($TCID_{50}$/ml) and is tested for sterility and identity. Consistency of production should guarantee a minimum titre in antigen yield. The virus suspension is then inactivated and tested for complete inactivation.

A series of preliminary studies was carried out in sheep using experimental batches of a monovalent proprietary vaccine containing a different BTV serotype (e.g. Zulvac 4), in order to determine the optimal quali-quantitative composition regarding adjuvants and antigen concentration. Initial immunogenicity and challenge experiments demonstrated a better performance of BTV4 vaccine antigen adjuvanted with a combination of $Al(OH)_3$ and saponin and provided some evidence for a correlation between the antigen concentration and reduction of viraemia in 2 months old vaccinated sheep. The higher the concentration of antigen, the lower was the viraemia. On the basis of these preliminary findings, some improvements in the manufacturing of the active ingredients were introduced and two concentrations of BTV4 antigen were tested for safety and immunogenicity in the presence of higher concentration of selected adjuvants and in comparison with two oily adjuvants. Challenge experiments were carried out using two different vaccine antigen concentrations and a selection of adjuvants (including $Al(OH)_3$ and saponin) in order to establish the best safety/immunogenicity ratio. Based on the results obtained from this final study, a concentration of vaccine antigen (according to pre-inactivation titre) and a quantity of 4 mg Al^{3+}/dose and 0.4 mg/dose of saponin respectively were selected. The information generated from these experiments was also taken into account for the development of the vaccine under application, as the same process is used for the manufacturing of the vaccine antigen, and the inactivation of BTV8 as for the BTV4 serotype. Also the same adjuvant(s) at the same concentration(s)/dose were used.

Zulvac 8 Bovis is blended on the basis of the pre-inactivation viral titre of the bulk antigen.
In order to establish the concentration of active ingredient to be used in the formulation of the bulk vaccine, a specific dose-response study was designed to test the safety and immunogenicity (in 2.5 month old calves) of the current vaccine at different concentrations of inactivated BTV8 antigen. A minimum antigen concentration (according to the pre-inactivation titre) was selected on the basis of this study taking into account that at this antigen concentration, 100% prevention of viraemia was achieved as well as a satisfactory level of local and systemic safety results. The vaccine is manufactured under GMP conditions, applying established manufacturing processes, using adjuvants and excipients well characterized and widely used for the production of veterinary vaccines. Control tests planned to be carried out during production and on the finished product should further guarantee a consistent quality profile of the vaccine. Regarding the Antimicrobial Preservative Efficacy (APE) the Applicant confirmed that a study is on going in order to demonstrate the efficacy of antimicrobial preservation during the product shelf life. The APE test is scheduled to cover each bottle size. In this study batches are tested for APE at different times after manufacturing.

Composition of the batches used in the clinical trials

Data were only provided from safety and efficacy trials carried out under laboratory conditions. This was acceptable based on the provisions in CVMP Reflection Paper on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue. Overall the relevance of the batches used to support the safety and efficacy studies was satisfactorily supported.

METHOD OF MANUFACTURE

A detailed flow chart of the whole manufacturing process of the vaccine was provided and considered satisfactory. The stages of the manufacturing process were described in sufficient details and all the operations stated to be conducted in conditions of sterility following established methods or sterile manipulation techniques. The process comprises 13 steps starting with the propagation of the working

seed virus (WSV) and ending 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.
More specifically, the virus vaccine is obtained after a number of passages on BHK-21 cells. After harvest the culture is inactivated and the inactivant neutralized at the end of the inactivation process. The bulk vaccine is further produced after blending pre-determined amount of inactivated and neutralized vaccine antigen, thiomersal, saline solution and adjuvants. All calculations of the volumes of the different components were described in sufficient details. These components are sequentially added to obtain the final blend. Two blending processes can be alternatively used by the Applicant. In both processes the same parameters and adjustments are performed to guarantee consistent batches. The equivalence of both processes has been satisfactorily demonstrated.

The filling operation is carried out in a laminar flow cabinet (class A), in class B environment. The filling operation has been validated. Glass bottles are washed and sterilised in validated cycles. Stoppers and seals are sterilized in autoclave in validated cycles.

The packing operation is conducted following a fully automatic process according to approved procedures for the packing of products, in accordance with the specific protocol.

Manufacture of vaccine antigen

Vaccine antigen is grown in BHK-21 cells. Parallel steps are carried out in order to obtain proper amplification of both BHK-21 cells and virus inoculum for the production of the active ingredient.

BHK-21 cell culture
The expansion of the initial cell seed is carried out in order to obtain the amount of cells needed for production. WCS cryotubes are thawed and then inoculated in a culture flask. From these culture flasks, the required subcultures are carried out. During the cell scale up process cultures are observed periodically, and its evolution (confluence), cell morphology and cell passage are recorded. The cell passage is recorded in order to monitor that the final passage used for virus production cannot be more that 20 from the MCS.

Virus growth
A Master Seed Virus (MSV) was constituted on BHK-21 cells, and stored frozen prior to vaccine production. The working seed virus (WSV) is expanded from the MSV into BHK-21 cells and also stored frozen.
In the antigen production process, the virus vaccine will produced from the WSV by passages into BHK-21 cells. Details were provided of optimal culture time triggering a virus harvest. During the antigen production process, samples are taken for titration, sterility and identity controls. Details were provided for all the controls carried out on the final antigen.
The Applicant indicated that the virus vaccine will be maximum a passage 5 from the MSV. The current culture vessels for BTV manufacture were provided. However, depending on future market demand, larger or various sizes may be used.

Manufacture of the inactivated and neutralized vaccine antigen

Final viral suspension is inactivated with BEI. The excess of inactivating agent is neutralised with sodium thiosulphate at the end of the inactivation process. Samples are taken from the inactivated and neutralized antigen to carry out in process controls.
The titre of the antigen before inactivation and the dilution factor that represent the additions in the inactivation and neutralization processes are taken into account in order to provide an indication of the theoretical titre of the inactivated and neutralized antigen. At routine industrial production, the vaccine is formulated to contain a defined amount of unconcentrated virus culture.
Provisions in the CVMP Reflection Paper on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue-EMEA/CVMP/IWP/105008/2007 allow to consider the virus titre before inactivation as appropriate for vaccine formulation.

Manufacture of the finished product
The bulk vaccine is prepared by blending pre-determined amounts of one or a mixture of several batches of inactivated and neutralized BTV-8 antigen with thiomersal, saline solution and adjuvants. The bulk vaccine can be stored at +5.0±3.0°C until the start of the filling operation. Primary packaging elements (bottles and closures) are sterilized by validated cycles. Once filled, bottles of vaccine are submitted to secondary packaging operations which are carried out using a fully automatic process. Finished product is stored at +5.0±3.0°C. The Applicant indicated the size of the antigen ingredient /vaccine batch and that, if necessary, more than one antigen batch may be used for vaccine blending. All the antigen batches used for vaccine bulk preparation must be in compliance with the approved specifications. The last step is the secondary packaging operations. Confirmation of GMP compliance was provided for relevant manufacturer batch protocols.

Validation studies

Validation of the manufacturing process

A number of studies were presented as part of the validation of the manufacturing process.

Inactivation kinetics
Several virus inactivation kinetics studies using batches of non-standard and standard sizes were carried out as part of the validation of the manufacturing process. Studies were GLP compliant and aimed to investigate the inactivation kinetics of BTV serotype 8. Studies using a BTV serotype 4 model were also presented. According to Eur. Ph. requirements the selected inactivating agent and the inactivation procedure shall be shown, under conditions of manufacture, to be capable to inactivate BTV8 within a time period equivalent to not more than 67% of the duration of the whole inactivation process. Based on the results of these studies the Applicant proposed a maximum pre-inactivation titre which was accepted.

Inactivation control
A GLP compliant study aiming to demonstrate the sensitivity of the inactivation control technique using serotype 8 of BTV was also provided. The limit of detection for BTV8 in the inactivation control is 0.08 $TCID_{50}$.

<u>*Conclusions:*</u> The Applicant provided evidence for the robustness of antigen production process by demonstrating the consistency in the manufacturing process and in the corresponding results obtained. Relevant data has been reported for 3 Batch Release Protocols from each manufacturing site. The above were considered satisfactory.

PRODUCTION AND CONTROL OF STARTING MATERIALS

STARTING MATERIALS LISTED IN A PHARMACOPOEIA

Starting materials of biological origin

None

Starting materials of non-biological origin

List of materials
Potassium chloride
Potassium dihydrogen phosphate
Sodium chloride
Disodium edetate
Water for injections

Disodium phosphate dodecahydrate
Sodium hydrogen carbonate
Gentamicin sulphate
Aluminum hydroxide
Thiomersal
Sodium hydroxide
Sodium Thiosulphate
Disodium phosphate dehydrate
Hydrochloric acid
Phenol red

The Applicant provided evidence of compliance to all relevant Eur. Ph., and presented updated certificate of analysis (CoA) for the above substances.

STARTING MATERIALS Not Listed in a Pharmacopoeia

Starting materials of biological origin

The following starting materials were assessed:

Starting material	Function
BHK-21 cells (clone 13)	Substrate for the replication of BTV-8
BTV serotype 8	Vaccine antigen (active ingredient)
Bovine calf serum (irradiated)	Source of protein for cell substrate
Pancreatic Digest of Casein produced from bovine milk (casein) and enzymes of porcine origin (ingredient of Glasgow MEM)	Glasgow MEM culture medium for BHK-21 cells used for manufacturing BTV8 antigen
Porcine trypsin	Used for the detachment of cells (BHK-21) from culture vessel surface
Saponin	Vaccine adjuvant
Gelatin, dextran (raw material for Microcarriers production)	Microcarriers used as support for BHK-21 cell growth

BHK-21 cells

The BHK-21 cell line is a baby hamster kidney cell line used as substrate for the production of BTV8 vaccine antigen. Once received by the Applicant from the Cell bank of Brussels, the cells underwent a series of passages in monolayer before a Master Cell Stock (MCS) was prepared. Current WCS is stated to have been prepared by performing subcultures from this MCS. MCS and WCS are stored at – 196°C in liquid nitrogen. Further details of MCS and WCS were provided in specific studies. According to the Applicant's records, the BHK-21 cell line was derived from the kidneys of five unsexed, 1-day-old hamsters, in March 1961, by I. A. Macpherson and M. G. P. Stoker (Virology 16:147, 1962). Details of the cell passage history before acquisition were not available. At Fort Dodge, the BHK-21 cell line is handled in a seed lot system. Details of the production of current MCS and WSC were provided. Certificates of Analysis and EDQM certificates of the batches of bovine serum used to produce the WCS and the MCS, respectively were provided.
Evidence was provided for the absence of cytogenetic differences between Master Cell Stock –MCS- and passage 20 from MCS. The absence of extraneous agents relevant to bovine and ovine species, and to the species of origin of the cell line (hamster) was tested. According to current EU legislation, the absence of viral contamination was checked by using general (CPE and HA) and specific testing. Moreover, ovine and bovine primary cells such as foetal lamb kidney (FLK) cells and primary bovine embryo kidney cells (PBEK), and continuous cell lines such as VERO (African Green Monkey Kidney) and Madin Darby Bovine Kidney (MDBK)-these cells sensitive to viruses pathogenic to the target species and to pestiviruses were also used to detect cytopathogenic and HA viruses and bovine/ovine specific viruses after the inoculation of MCS and WCS. The results of such a testing,

carried out in order to fulfil the requirements of current EU legislation, were reported and were acceptable.

BTV8 antigen

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. A flow chart showing the initial passages which the virus strain underwent after isolation was provided.
Sterility and absence of mycoplasma contamination in the MSV and WSV according to Eur. Ph. was demonstrated. MSV and WSV are stored frozen at a T° lower or equal to –70°C ± 10°C.
The Applicant provided evidence that the MSV is negative to cytopathic viruses, haemadsorbing viruses and that it is free of the following specific agents: Adenovirus, Bornavirus, FMDV, BRSV, Louping ill virus, EHDV, Akabane virus, Rabies Virus, BVDV, BLV, Brucella, Mycobacterium and *Chlamydia*. It was shown that controls on MSV and WSV were conducted according to relevant EU legislation using validated techniques. Concerning the absence of contamination by Rift Valley fever virus, Nairobi sheep disease virus and Ross River Virus the Applicant indicated that as the MSV is not derived from an isolate coming from the relevant countries/continents to these viruses they have not been performed.
Information was also provided that indicated that the BTV 8 strain used in the Zulvac 8 vaccine was isolated from the blood of a 2-year-old Belgian sheep and that before exportation, 3 sheep from the same flock were tested for scrapie, in 2005, 2006 and 2007, and were found negative at each time point testing. The negative TSE status of the farm of the sheep at the origin of the vaccine was also confirmed.

***Bovine calf serum**, irradiated*

Bovine calf serum is used as component of cell culture medium. Assurance that the donor animals comply with the regulations concerning TSEs including a certificate of analysis of one batch of bovine calf serum provided by the official supplier and a corresponding EDQM certificate of suitability. Confirmation was provided of the specifications for each batch of bovine serum. Purity tests and γ-irradiation are used as complementary measures to achieve a high security level against potential contamination. Overall the principle adopted for extraneous agents testing was acceptable. The validation of the irradiation method was provided.

Pancreatic Digest of Casein produced from bovine milk (casein) and enzymes of porcine origin (ingredients of Glasgow MEM culture medium)

A CoA provided by the supplier together with details of source/origin of raw materials mentioning that this contains pancreatic digest of casein obtained from fermentation. Fermentation medium contains casein produced from bovine milk and porcine enzymes. A statement was also provided that the milk is collected from healthy animals in the same conditions as milk collected for human consumption.

(Porcine) Trypsin

Porcine trypsin is manufactured from pancreas of swine and contains bovine milk lactose. The statement of origin should guarantee that the bovine milk is collected from healthy animals in the same conditions as milk collected for human consumption. A series of corresponding CoAs, specifications and methods of analysis were provided. The Applicant confirmed that testing is carried out on each batch of trypsin.

Saponin (purified)

Saponin is a liquid substance of vegetable origin. A CoA was provided from the supplier regarding the control of general characteristics for saponin including appearance and identity, saponin concentration (by HPLC), dry matter and haemolysing saponin content. The Applicant provided satisfactory evidence in order to demonstrate that the irradiation process is not necessary, as animal viruses could not be found in the product.

Microcarriers biological origin raw materials

Can be alternatively used in standard manufacturing. The quality of microcarriers has been defined and specification monographs for this raw material have been provided. Gelatin and dextran are raw materials used for the manufacture of microcarriers:

Gelatin (of porcine origin)

One of the raw materials used for the manufacture is gelatine, which is processed from pigskin. Currently, materials from porcine origin do not carry any risk of transmitting TSE, according to the Note for Guidance EMEA/410/01-Rev 2.

Dextran

Dextran is also used for the manufacture of the microcarriers. Skimmed milk powder derived from bovine milk fitted for human consumption from USA is used for the manufacture of Dextran. Milk derivatives are excluded from the Note for Guidance EMEA/410/01-Rev 1 as long as the milk is sourced from healthy animals under the same conditions as milk considered fit for human consumption. TSE statement from the supplier was provided. The freedom from contamination with extraneous agents was demonstrated.

Starting materials of non-biological origin

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

Starting material	Used for preparation of/ function
Bromoethylamine Hydrobromide (BEA)	PI V8 inactivating agent
Dimethyl sulfoxide (DMSO)	Cryoprotector

Bromoethylamine Hydrobromide (BEA): is prepared from raw materials (2-bromoethylamine hydrobromide and Sodium hydroxide) that are not susceptible to contamination. The solution is prepared under aseptic conditions and added to the culture in the same manner.
Dimethyl sulfoxide (DMSO): The Applicant clarified that DMSO supplied by Sigma Aldrich does not comply with Eur. Ph. Monograph 2005:0763. This was justified. However it was noted that as the amount of DMSO in the final product is negligible as a consequence of its use in the preparation of MCS and WCS, therefore any risk deriving from non compliance should be of negligible effect.

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 have been provided to support the quality of the following media: Glasgow MEM, PBS, Saline solution, Trypsin solution-cells; Trypsin 0.05% solution; EDTA 5% solution; Sodium hydrogen carbonate 7.5% solution; Phenol red solution; Sodium hydroxide 0.2N, 1N, 6N solutions; 0.1M BEI solution; 1M Sodium thiosulphate solution; Thiomersal 10% solution; saponin 1% solution; microcarrier suspension. These were all found acceptable.

SPECIFIC MEASURES CONCERNING THE PREVENTION OF THE TRANSMISSION OF ANIMAL SPONGIFORM ENCEPHALOPATHIES

An assessment was conducted in order to demonstrate that the risk for transmission of TSE due to the starting materials of animal origin used in the manufacturing of this vaccine is minimal. The minimisation of risk is achieved by: a) the documented and recorded sourcing of animals (animal-derived material of known and controlled origin), b) the nature of animal tissues used in

manufacturing (low or no detectable infectivity), c) the production processes, and d) the negligible risk posed by a series of factors which would likely lower the risk if any. These factors include the high dilution of the materials used, the route of administration and the maximum number of dosage injected. A risk assessment (RA), certification of suitability and declaration of conformity were provided as appropriate, specifically, for the MCS and WCS of the BHK-21 cells; for the MSV and WSV, for the bovine calf serum; for the casein of bovine origin and the enzymes of porcine origin contained in Glasgow MEM; for the trypsin and for the microcarriers raw material's of animal origin. The Applicant also submitted certificates that stated the exclusion of the presence of any substance of animal origin in the production of saponin adjuvant. Satisfactory justifications were provided for the use of material from a GBRIII level Country (bovine serum sourced from US).

CONTROL TESTS DURING PRODUCTION

The Applicant clarified the production parameters of the active ingredient that are monitored during the process and the rationale for their choice. A detailed flow chart was provided, showing at which stage of production controls are carried out on the following intermediate products: antigen passage 1 ; antigen passage 2; antigen passage 3; inactivated/neutralized antigen and bulk vaccine.

Controls include the following tests:

- Antigen passage 1: Titration
- Antigen passage 2: Sterility and titration
- Antigen passage 3: Sterility, titration and identity
- Inactivated and neutralized antigen: Sterility, inactivation control, sodium thiosulphate contents
- Bulk vaccine: Sterility, absence of Aujeszky's disease virus, absence of Pestivirus, inactivation control

The above tests were described in detail and they were all found satisfactory.

CONTROL TESTS ON THE FINISHED PRODUCT

The controls on the finished products were described in sufficient details. The methods, frequency, pass criteria for the tests were acceptable.

Controls include the following tests:

- General characteristics of the finished product: Appearance, volume, pH
- Identification and assay of active substance: Identity, *in vivo* potency test in mice.

In vivo potency test:
In order to confirm that each batch of Zulvac® 8 Bovis formulated on the basis of the virus titre measured before inactivation is efficacious in the target species, the Applicant has developed an *in vivo* batch potency test in mice. The Applicant will use this test for the release of the commercial batches of Zulvac® 8 Bovis vaccine. Results from validation studies performed to support this Batch Potency Test were provided and were found satisfactory. The quantitative composition of the vaccine based on the test is expressed in relative potency (RP) with regard to a reference vaccine that was shown efficacious in lambs. The specification of a potent batch was ≥ 1 when compared to the reference vaccine.

- Identification and assay of adjuvants and excipients: aluminium hydroxide content and thiomersal content. The Applicant has also developed and validated a method for the determination of saponin. The method is going to be implemented by the Applicant at manufacturing sites.
- Sterility and purity test: sterility and absence of extraneous BTV
- Safety test in the target species

Consistency of production

The Applicant provided satisfactory data that demonstrates the consistency of production and the equivalence between all the manufacturing sites. The Applicant was requested as a post-approval commitment to provide results from 3 consecutive full size production batches.

STABILITY

Active substance
Twelve months after its production, a BTV-8 antigen was blended to produce a vaccine. The Applicant confirmed that this vaccine batch will follow the stability program for which the protocol is already submitted to cover the 24 months shelf life. The Applicant will test the potency of this vaccine according to the mice batch potency test.

Finished product
A real-time-stability-study protocol aiming to demonstrate a 24 months shelf life of the finished product was provided**.** An interim report was submitted showing stability of one batch at 12 months and one batch at 3 month. The conducted tests showed that for 12 months the vaccine was stable. However in this study the new potency test in mice was not used. The Applicant committed to test the potency of the above batches in mice**.** As a support to the stability claim of the vaccine Zulvac 8 Bovis, the Applicant further provided the stability data obtained to date of the vaccines Zulvac 4 which show a 12-months shelf life for the finished product.

The CVMP concluded that in the absence of final stability data a maximum shelf life of 12 months can be granted based on the provisions in the CVMP Reflection Paper on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue-EMEA/CVMP/ /IWP/105008/2007.

OVERALL CONCLUSION ON PART II ASSESSMENT

All the data and clarification provided by the Applicant can be considered sufficient for granting a marketing authorisation under exceptional circumstances, when taking into account the benefit-risk balance for BTV serotype 8, and when considering the epidemiological situation in the EU.
In this context given that:

- a batch with minimum antigen content was shown efficacious on cattle,
- the production process allows production of consistent batches, with now a reliable batch potency test

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

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

3. SAFETY ASSESSMENT

INTRODUCTION AND GENERAL REQUIREMENTS

Zulvac 8 Bovis is a conventionally produced, liquid and ready-to-use, BEI inactivated vaccine, adjuvanted with aluminium hydroxide ($Al(OH)_3$) and saponin. The final batches of the vaccine are formulated in a 2 ml dose containing, an inactivated BTV 8 strain (blended at a target concentration based on the pre-inactivation titre) and a consistent amount of 4 mg of Al^{3+} and 0.4 mg of saponin. Thiomersal (0.2 mg/dose) is added as preservative, the vaccine being presented in multi-dose bottles.

A 2 ml dose is recommended to be administered by intramuscular route to cattle (including pregnant animals) to reduce, in emergency situations, the viraemia established in animals infected by serotype 8 strains of BTV. The basic vaccination schedule consists of one injection given from a minimum of 3 months of age followed by a second injection given 3 weeks later. Revaccination is recommended every 6 months.
Current European legislation, stipulates that studies should be performed to demonstrate the safety of a vaccine for target animals of the youngest age for which the vaccine is intended to be used, and, if the vaccine is intended to be used in breeding animals, examination of the reproductive performances should also be carried out. The safety of the administration of an overdose of the vaccine needs also to be investigated in animals of the target species. According to the CVMP Reflection Paper on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue-EMEA/CVMP/IWP/105008/2007) representative experimental batches or standard production batches of the vaccine can be used in safety studies. The reflection paper also allows that data generated from other vaccines of similar composition (in terms of excipients and adjuvants) in the same or a similar range of target species, can be used to fulfil safety requirements. In the same document, field trials are not strictly required.

The Applicant provided results from safety studies carried out in target animal species of the minimum age recommended for vaccination, using a movovalent vaccine (serotype 1) and a bivalent vaccine (serotypes 1 and 8) containing the same amount of adjuvant(s)/excipients. All vaccines used in studies presented below were blended at maximum antigen concentration based on the titre before inactivation.

LABORATORY TRIALS

The local and general tolerance to vaccination was studied after each administration of the vaccine. The standard parameters used to support the safety profile of the vaccine are listed below:

- Clinical signs after vaccination
- Impact on body temperature (Tº)
- Local reactions (LR)
- Post mortem examination of injection sites

Safety of the administration of one dose / Safety of the repeated administration of one dose.

Efficacy and safety study of the vaccine Zulvac 8 Cattle formulated at different concentrations of BTV, serotype 8, in 2.5 month old calves

Objective/Methodology: To evaluate the safety (and the efficacy) of the intramuscular injection of four experimental vaccine preparations formulated with different payloads of BTV8 antigen, and standard amounts of adjuvants (representing standard production of the actual vaccine under application). The vaccine was administered at 2m/dose following a primary vaccination course in calves of minimum age (GLP compliant study)

Animals

Calves 2.5 months old, were randomly distributed in 4 groups (1 to 4) of ten animals (vaccinates) each and 1 group (5) of 10 controls. A good health status was a criterion for inclusion, as well as sero-negative status of calves against BTV.

Materials: Vaccine/Placebo

Four experimental vaccines (A to D) were formulated with different payloads of BTV8 antigen starting with vaccine A which had the highest antigen load (maximum standard dose) and decreasing to D with the lowest. The amount of aluminium hydroxide Al^{3+} and saponin was the standard used per 2 ml dose. Saline solution q.s. to 2ml and 0.2 mg of thiomersal were also added as component of the final 2ml dose of the vaccine. Each vaccine preparation was allocated to an appropriate group.

Administration route and vaccine scheme

On D0, each calf of group 1, 2. 3 and 4 was intramuscularly (im) injected in the right neck area with 1x2ml dose of the corresponding vaccine preparation. About 3 weeks later (from D19 to D21), these animals were revaccinated under the same conditions (one-2ml dose, im). 10 calves were allocated in group 5 and acted as control for the challenge experiment. These animals remained untreated.

Observation scheme and post-vaccination follow-up (limited to safety)
After each vaccination, and for 14 days thereafter, calves were monitored for the appearance of any systemic reactions. Rectal temperature was measured on the day before each administration of vaccine, four hours after each administration of vaccine, and then daily for the 2 days following each administration of vaccine. Injection sites were carefully inspected for 14 days after each vaccination to detect any local reaction.
Post-vaccination follow-up (immunogenicity)
Blood samples were collected before 1st vaccination (D0), approximately 2 weeks after 1st vaccination (D16); about 2 and 3 weeks after revaccination challenge, in order to detect antibodies against BTV 8 (these data was not presented; testing is stated to be performed at the Applicant's R&D Laboratory).
Results (limited to safety)
No systemic reactions were reported to have occurred in any of the vaccinated animals. Results of measurement of rectal temperature and detection of local reactions after vaccination and revaccination were only reported for animals of groups 1 (vaccine A) and 2 (vaccine B). No statistically significant differences were ever recorded among values of rectal T° measured in vaccinated and control calves.
Conclusions (on safety)
In 2.5 month old calves, vaccination/revaccination with Zulvac 8 Bovis do not induce systemic or local reactions at the injection site.

1) Safety study of the administration of a repeated single dose of Zulvac®1 Bovis in 2.5 to 3-month-old calves.
The objective of the study was to verify the safety of the administration of the repeated administration of one dose of a monovalent inactivated BTV-1 vaccine (Zulvac® 1 Bovis) in 2.5 to 3-months-old calves.
In this study calves were vaccinated intramuscularly (i.m.) three times, 3 weeks apart (D0-D21-D42), with one dose (2 ml) of Zulvac® 1 Bovis. Animals with similar characteristics were treated with a placebo substance consisting of PBS. The animals were monitored for the appearance of anaphylactic reactions in the hours immediately after the vaccination. Recording the calves rectal temperature was carried out the day before vaccination, the day of vaccination just before the vaccination, 4 hours after the administration of the vaccine and thereafter for the following 4 days. Recording local reactions at the inoculation sites during 14 days after each vaccination was carried out together with daily observation of the calves' health conditions. Three weeks after the 3rd vaccination all the calves were euthanized in order to perform a macroscopic and/or microscopic examination of the injection sites.
Results
The calves did not manifest any anaphylactic reactions after the 1st, 2nd and 3rd administration of the vaccine. The vaccine did not induce any rectal temperature increase after vaccinations.
None of the calves presented any *in vivo* local reactions (swelling and/or reddening) at the injection sites after vaccinations. None of the calves presented neither macroscopic nor microscopic tissue lesions at the inoculation sites.
Conclusions:
The results of this study are considered supportive to the safety of Zulvac 8 Bovis vaccine.

2) Safety study of the administration of a repeated single dose of Zulvac®1+8 Bovis vaccine in 3-month-old calves.
The objective of the study was to verify the safety of the administration of the repeated administration of one dose of a bivalent inactivated BTV1/BTV-8 vaccine (Zulvac®1+8 Bovis) in 3-months-old calves.
In this study calves were vaccinated intramuscularly (i.m.) three times, 3 weeks apart (D0-D21-D42), with one dose (2 ml) of Zulvac® 1+8 Bovis. Animals with similar characteristics were treated with a placebo substance consisting of PBS. The animals were monitored for the appearance of anaphylactic reactions in the hours immediately after the vaccination. Recording the calves rectal temperature was carried out the day before vaccination, the day of vaccination just before the vaccination, 4 hours after the administration of the vaccine and thereafter, for the following 4 days. Recording local reactions at the inoculation sites during 14 days after each vaccination was carried out together with daily observation of the calves' health conditions. Three weeks after the 3rd vaccination all the calves were euthanized in order to perform a macroscopic and/or microscopic examination of the injection sites.

Results
The calves did not manifest any anaphylactic reactions after the 1st, 2nd and 3rd administration of the vaccine. The vaccine did not induce any rectal temperature increase after 1st and 3rd vaccinations.
Twenty four hours after 2nd vaccination, a transient but statistically significant increase of rectal temperature of 0.4°C was recorded in the vaccinated animals, on day 2 after vaccination, rectal temperature had normalized. None of the calves presented any *in vivo* local reactions (swelling and/or reddening) at the injection sites after vaccinations. None of the calves presented either macroscopic or microscopic tissue lesions at the inoculation sites.
Conclusions:
The results of this study were considered supportive to the safety of Zulvac 8 Bovis

Safety of an administration of an overdose

1) Study on the safety of the administration of an overdose of the vaccine Zulvac® 1 Bovis in 2.5-months-old calves.
The objective of the study was to verify the safety of the administration of an overdose of a monovalent inactivated BTV-1 vaccine (Zulvac® 1 Bovis) in 2.5-months-old calves.
In this study calves were vaccinated i.m. with one overdose (4 ml) of Zulvac® 1 Bovis. Animals with similar characteristics were treated with a placebo substance consisting of PBS. The animals were monitored for the appearance of anaphylactic reactions in the hours immediately after the vaccination. Recording the calves' rectal temperature was carried out the day before vaccination, the day of vaccination just before the vaccination, 4 hours after the administration of the vaccine and thereafter, for the following 4 days. Recording local reactions at the inoculation sites during 14 days after each vaccination was carried out together with daily observation of the calves' health condition.
Results
The calves did not manifest any anaphylactic reactions or rectal temperature increase after the administration of a vaccine overdose. The inoculation of a double dose of the test vaccine did not provoke the appearance of local reactions (swelling and/or reddening at the site of injection).
Conclusions
Anaphylactic reactions or vomiting are not induced by the administration of a double dose of the tested vaccine. Neither increase of rectal temperature or local reactions at injection site are induced in the vaccinated animals after the administration of a double dose of the tested vaccine. In general, an acceptable level of safety was demonstrated for the tested vaccine when administered in a 2x 2ml/dose to calves of minimum age recommended for vaccination.

2) Safety of the administration of an overdose of the vaccine Zulvac® 1+8 Bovis in 3 month-old calves
The objective of the study was to verify the safety of the administration of an overdose of a bivalent inactivated BTV1/BTV-8 vaccine (Zulvac® 1+8 Bovis) in 3-months-old calves.
In this study calves were vaccinated i.m. with one overdose (4 ml) of Zulvac® 1+8 Bovis. Animals with similar characteristics were treated with a placebo substance consisting of PBS. The animals were monitored for the appearance of anaphylactic reactions in the hours immediately after the vaccination. Recording the calves' rectal temperature was carried out the day before vaccination, the day of vaccination just before the vaccination, 4 hours after the administration of the vaccine and thereafter, for the following 4 days. Recording local reactions at the inoculation sites during 14 days after each vaccination was carried out together with daily observation of the calves' health condition.
Results
The calves did not manifest any anaphylactic reactions after the administration of a vaccine overdose. Regarding rectal temperatures, the administration of a double dose of the test vaccine induced a slight and transitory but significant increase in the mean rectal temperature of 0.7ºC in the vaccinated calves 24 hours after the administration. On day 2 after vaccination, rectal temperatures had normalized. The inoculation of a double dose of the test vaccine did not provoke the appearance of local reactions (swelling and/or reddening at the site of injection).
Conclusions
The inoculation of a double dose of the test vaccine did not provoke the appearance of local reactions (swelling and/or reddening at the site of injection).

Examination of reproductive performance
No laboratory data are available on reproductive performance in cattle. However, the Applicant has committed to conduct a safety study in dairy cows at different phases of gestation in order to prove that the vaccine does not affect milk production and reproductive parameters. A number of studies performed in pregnant ewes with a vaccine of similar composition but of serotype 4 were presented, the results obtained were supportive.

Examination of immunological functions
No specific study was carried out in this respect as no negative influence on the immune response is expected due to vaccination. There is no evidence to support an impairment of the immune system due to the vaccination.

Special requirements for live vaccines
Not applicable

Interactions
Interaction with other veterinary medicinal products has not been investigated.
A recommendation for not mixing the vaccine with other IVMPs has been included in SPC.

FIELD STUDIES

Data from field studies were not provided. In light of the current requirements of the CVMP Reflection Paper on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue. (EMEA/CVMP/ /IWP/105008/2007) on field studies this approach was acceptable. The Applicant also made reference to safety field studies that were conducted in Germany (J Gethmann, *et al,* Comparative safety of three inactivated BTV-8 vaccines in sheep and cattle under field conditions, Vaccine, 2009) - using Zulvac® 8 Bovis vaccine. In these studies the safety of the vaccine was demonstrated. The parameters used to assess safety were monitoring local reactions at the injection site, general reactions, reproductive parameters (abortions, normal birth, teratogenic effects, etc.) and milk production. The above bibliographical reference was noted.

ENVIRONMENTAL RISK ASSESSMENT (ECOTOXICITY/USER SAFETY)

A Phase I assessment of risk was performed according to the relevant guideline EMEA/CVMP/074/95. The final product contains no components which may exert a toxic effect and there are no pharmacologically active components included in this vaccine. Phase 1 assessment provided evidence that there would be no potential risk for the global environment.

No negative impact on public health or on the environment can be identified in light of the nature of the vaccine, in particular of the antigen (inactivated) and adjuvant(s) (appearing to be pharmacologically inert substances). Additionally, no special concern is posed by the final product in light of the safety of packaging, of the limited number of injections and of the maximum quantity administered to animals, of the route and of the method of administration. Consequence and level of risk are practically nil, thus justifying the absence of phase 2 assessment.

RESIDUE ASSESSMENT

The Applicant has provided acceptable justifications for omitting specific studies on residues. The vaccine contains adjuvants, excipients and preservatives with well known properties and characteristics.

MRLs

The following substances are included in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Aluminium hydroxide gel	All food producing species	
(Quillaia) Saponin	All food producing species	
Thiomersal	All food producing species	For use only as preservatives in multidose vaccines at a concentration not exceeding 0.02 %
Potassium chloride	All food producing species	
Potassium dihydrogen phosphate	All food producing species	
Disodium hydrogen phosphate dodecahydrate	All food producing species	
Sodium chloride	All food producing species	

Water for injections is considered as not falling within the scope of Council Regulation (EC) 470/09

OVERALL CONCLUSIONS ON SAFETY

The provision of additional safety data demonstrated the safety of the vaccine in cattle of minimum age. The safety study in pregnant cows is awaited. Overall, the safety profile of Zulvac 8 Bovis vaccine was demonstrated. The potential for any adverse effects following the administration of the vaccine under the recommended conditions of use is adequately reflected in the relevant section of the SPC.

4. EFFICACY ASSESSMENT

Introduction and general requirements

Zulvac 8 Bovis is recommended for the active immunisation of cattle from 3 months of age for the prevention of viraemia caused by bluetongue virus, serotype 8. A 2 ml dose is recommended to be administered by intramuscular route to cattle; the basic vaccination schedule consists of one initial injection given from a minimum of 3 months of age followed by a second injection given 3 weeks later. Onset of immunity is 25 days after the completion of the basic vaccination course. The duration of immunity (DoI) has not been fully established yet but interim results support one of at least 6 months. Although safety and efficacy studies were not performed in breeding animals, its use is also recommended during pregnancy and lactation, in line with the approach taken in similar BTV8 centralised vaccine applications. The absence of any investigation of the influence of maternally derived antibodies (MDA) on vaccine efficacy has not been studied yet, such an omission being reflected in a statement included in SPC. Field trials were not strictly required for this type of application; the Applicant provided bibliographical data related to them. A DIVA strategy has not been implemented yet.

LABORATORY TRIALS

In order to establish a correlation between antigen load (expressed in terms of virus titre before inactivation) and vaccine efficacy, one laboratory trial was carried out and later a second one of supporting nature. Batches of experimental vaccines were formulated to contain decreasing amount of vaccine antigen and were tested in a dose/response study carried out in BTV-8 free calves of minimum age. The efficacy induced by the different concentrations of vaccine antigen was challenged against

the appearance of clinical signs and viraemia after of the inoculation of an infective dose of a homologous BTV 8 strain isolated from a recent outbreak of BTV8 in Belgium. Viraemia was analysed by BTV specific quantitative real time RT-PCR assay.

Validation of challenge model

The focus challenge model, was to inoculate an amount of virus so that all control animals become viraemic during the study (the viraemia started approximately 3 to 5 days post infection and is present at least until 27 days post infection that is the finishing date of the study), since the target of the vaccine is "prevention of viraemia in the vaccinated animals". The Applicant presented results of all the immunogenicity studies carried out where it is observed that in all the challenge doses tested, the 99% of the animals become viremic after challenge. The Applicant also provided the results of additional studies aiming to investigate the correlation between the quantity of challenged virus and the detection of viral genome. The challenge model was validated based on previous experiments with BTV-4 and BTV-1, in which under the conditions at this concentration all the animals inoculated were viraemic.
From the overall conclusions derived from the experiments conducted until now, the challenge model can be considered as validated since it was demonstrated that animals inoculated become viraemic and consequently that a comparative evaluation can be made of the Ct values of vaccinated versus non-vaccinated to claim prevention or reduction of viraemia in vaccinated animals.
Reference was made to the pathogenesis of BTV in order to justify why non-viraemic animals presented clinical signs of infection (this phenomenon was reported to have been observed also in the field).

1) Efficacy and safety study of the vaccine Zulvac 8 Bovis formulated at different concentrations of BTV, serotype 8, in 2.5 month old calves
Objective
The objective of the study was to evaluate the efficacy of four experimental Zulvac 8 Bovis vaccine preparations formulated with different payloads of BTV8 antigen, and standard amounts of adjuvants. The vaccine was administered at two doses of 2 ml, administered 3 weeks apart, in calves of minimum age (GLP compliant study). The efficacy of the vaccines was evaluated based on their capacity to reduce or prevent viraemia after a BTV-8 challenge carried out 25 days after the second vaccination.

Experimental design
Calves 2.5 months-old, were randomly distributed in 5 groups as follows: 4 groups (1 to 4) of calves vaccinated and revaccinated with the four experimental Zulvac 8 Bovis vaccine preparations and one group (5) of controls. A good health status was certainly a criterion for inclusion, as well as seronegative status of calves against BTV. On D0, each calf of groups 1, 2, 3 and 4 was intramuscularly (i.m.) injected with a 2 ml dose of the corresponding vaccine preparation. About 3 weeks later (D19), these animals were revaccinated under the same conditions. Calves allocated in group 5 acted as control for the challenge experiment and remained untreated.
Blood samples were collected before 1st vaccination (D0), approximately 2 weeks after 1st vaccination (D16), about 2 and 3 weeks after revaccination (D32 and D40) and on D44 before challenge, in order to detect SN and ELISA antibodies against BTV 8.
A challenge with a virulent BTV8 field isolate originated from an outbreak in Belgium in 2006 was carried out 25 days after 2nd vaccination (e.g.D44). From the day of challenge, daily starting 3 days after and up to D14, on D17, 19 and 24 after challenge, animals were monitored for the appearance of major clinical signs reported to being observed during BTV8 infection (these signs including increase of rectal T°, nasal discharge, watering, coughing, dyspnoea, limping, prostration and mortality). Blood samples necessary for assessing viraemia (by the evaluation of presence of BTV genome by RT-PCR-testing) were collected on the day of challenge (D44) and then 3, 5, 7, 10, 13, 17, 20, 24 and 27 days after. At the end of the study all calves were euthanized and incinerated. The Applicant provided supportive evidence for the RT-PCR method used to define the viraemic status of animals which demonstrated that it was reliable enough to be used as detection method. Real time RT-PCR technique was carried out by the Spanish Reference Laboratory for BTV virus: Laboratorio Central de Veterinaria, Algete, Madrid. Definition of protection: Consistent absence of viral load detectable by

real time RT-PCR in all the vaccinated animals during the monitoring period of 4 weeks, defining viral load detectable by real time RT-PCR as the one that provides as a result a Ct value lower than 36.0.

Results

Clinical signs

One control animal died before challenge. Vaccination and revaccination did not prevent the occurrence of clinical manifestations in animals of any group.

Based on analysis of the clinical signs before and after the challenge the Applicant supported the view that the clinical signs observed were very likely due to respiratory infections rather than bluetongue virus infection. In the studies conducted at laboratory level, it was verified that the BTV-8 challenge used was able to replicate very well in target tissues (in 99% of the unvaccinated/challenged animals viral genome can be detected - secondary viraemia). However, the strain's capacity to induce clinical signs is very low (low pathogenicity strain). The only parameter that can be noticed is that in unvaccinated animals, as a consequence of viral replication in target tissues (inflammation, damage of tissues, etc.) hyperthermia can be detected.

Viraemia:

Viral genome was not detected in any of the calves vaccinated with the experimental vaccine preparation containing the highest payload of BTV8 antigen (100% prevention of viraemia).

Viral genome was detected in the 100% of the control unvaccinated calves from 5-7 days post infection.

Conclusions

Based on the results of this study, the administration of two doses of the experimental vaccine Zulvac 8 Bovis used in Group 1 resulted in a total protection of calves vaccinated at a minimum age, against viremia. The Applicant agreed to formulate the vaccine at this dose which appeared to be the one with the optimum results.

2) Efficacy study of Zulvac 8 Bovis vaccine in 2.5 month old calves

Objective

The objective of the study was to evaluate the efficacy of four experimental Zulvac 8 Bovis vaccine preparations formulated with different payloads of BTV8 antigen, and standard amounts of adjuvants.

This new study was designed to compare and support the results of the previous study were there was not a good correlation between the different vaccine concentrations tested and the real-time RT-PCR results.

The vaccine was administered at two doses of 2 ml, administered 3 weeks apart, in calves of minimum age (GLP compliant study).

The efficacy of the vaccines was evaluated based on their capacity to reduce or prevent viraemia after a BTV-8 challenge carried out 25 days after the second vaccination.

Experimental design

Calves 2.5 months-old, were randomly distributed in 5 groups as follows: 4 groups (1 to 4) of calves vaccinated and revaccinated with the four experimental Zulvac 8 Bovis vaccine preparations and 1 group (5) of controls. A good health status was certainly a criterion for inclusion, as well as seronegative status of calves against BTV.

On D0, each calf of group 1, 2 3 and 4 was intramuscularly (im) injected with a 2 ml dose of the corresponding vaccine preparation. About 3 weeks later (D21), these animals were revaccinated under the same conditions. Calves allocated in group 5 acted as control for the challenge experiment and remained untreated.

Blood samples were collected before 1st vaccination (D0), 3 weeks after 1st vaccination (D21); 2 and 3 weeks after revaccination (D34 and D41) and on D46 before challenge, in order to detect SN and ELISA antibodies against BTV 8.

A challenge with a virulent BTV8 field isolate originated from an outbreak in Belgium in 2006 was carried out 25 days after 2nd vaccination (e.g.D46). Two ml of the virulent challenge material was inoculated in the jugular vein of each animal. Blood samples necessary for assessing viraemia (by the evaluation of presence of BTV genome by RT-PCR-testing) were collected on the day of challenge (D46) and then 3, 6, 8, 10, 13, 16, 22, 24 and 27 days after. At the end of the study all calves were euthanized and incinerated. The Applicant provided supportive evidence for the RT-PCR method used to define the viraemic status of animals which demonstrated that it was reliable enough to be used as

detection method. Real time RT-PCR technique was carried out by the Spanish Reference Laboratory for BTV virus: Laboratorio Central de Veterinaria, Algete, Madrid. Definition of protection: Consistent absence of viral load detectable by real time RT-PCR in all the vaccinated animals during the monitoring period of 4 weeks, defining viral load detectable by real time RT-PCR as the one that provides as a result a Ct value lower than 36.0.

Results

Viraemia:

There was a good correlation between the amount of BTV8 antigen contained in the four experimental vaccine preparations tested and the detection of viral genome (from the 100% to the 25%).

Viral genome was detected in the 100% of the control unvaccinated calves from 3-6 days post infection.

Conclusions

The study demonstrated a good correlation between the different vaccine concentrations tested and the real-time RT-PCR results and a significant protection of the vaccinated animals.

The administration the experimental vaccine Zulvac 8 Bovis used in Group 1 resulted in a total protection of calves, vaccinated at a minimum age, against viremia.

The CVMP considered that the Applicant's conclusion on the experimental batches of vaccine used in this study were sustainable. The study clearly allowed the evaluation of the challenge conditions and the assessment of viraemia.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

The efficacy of the vaccine in the face of Maternally Derived Antibodies (MDA) has not been investigated. A warning is included in the relevant section of SPC.

Duration of Immunity

The Applicant indicated that no final data were available at present concerning the DoI, but a study is ongoing. For this study, the Applicant provided the timelines and submitted interim results. In the mean time, the absence of DoI data has been clearly reflected in relevant section of SPC.

As mentioned above, interim results were presented from a study conducted in calves. The study is presented below:

1) Duration of immunity study of Zulvac® 8 Bovis vaccine in calves (Interim Report)

Objective

The objective of the first part of the study was to verify if the administration of Zulvac 8 Bovis vaccine was able to prevent viraemia (no detection of viral genome by real time RT-PCR technique during 27 days post challenge) in calves challenged 7 months post vaccination. In the second part of the study, the remaining calves will be challenged 1-year post vaccination. Three experimental Zulvac 8 Bovis vaccine preparations formulated with different payloads of BTV8 antigen, and standard amounts of adjuvants were tested in the study.

Experimental design

Calves 2.5-3 months old, were randomly distributed in 4 groups as follows: 3 groups (G1, G2 and G3) of calves vaccinated and revaccinated with the three experimental Zulvac 8 Bovis vaccine preparations and 1 group (G4) of controls. A good health status was certainly a criterion for inclusion, as well as seronegative status of calves against BTV.

On D0, each calf of group 1, 2 and 3 was intramuscularly (i.m.) injected with a 2 ml dose of the corresponding vaccine preparation. About 3 weeks later (D21), these animals were revaccinated under the same conditions. Calves allocated in group 5 acted as control for the challenge experiment and remained untreated.

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 (seroneutralizing antibodies) after vaccination. On D232, calves from G1 and G2 and from G4 were submitted to a virulent challenge with BTV8 strain. Blood samples were taken from all animals on D0, 2, 5, 7, 9, 12, 15, 19, 23 and 27 after challenge (clinical signs were also recorded at the same time) for the evaluation of the presence of BTV genome by a real time RT-PCR. The technique was validated through an inter-laboratory validation assay for the quantitative detection of BTV genome. Protection was defined as a

constant absence of viral load detectable by real time RT-PCR (Ct value ≥ 36.0) in all vaccinated animals during the monitoring period of 4 weeks.

Results

Serology:

The evolution of seroneutralizing antibodies against BTV8 from vaccination until 7 the months challenge (D202) was provided. The SN geometric mean titres - GMT- were rather low and declined even at negative level at the time of challenge (on D232 a GMT of 3.8 and <2 were recorded in calves of G1 and G2, respectively). Control calves remained negative (GMT <2) at all the bleeding time points.

Clinical signs:

No statistically significant difference in the rectal temperature was recorded between vaccinated and control animals at any time points. There were no statistically significant differences in the clinical sign scores recorded between vaccinated (G1 and G2) and control (G3) calves; overall, just mild and non-specific clinical signs were recorded after challenge.

Viraemia:

Viral genome was not detected in any of the vaccinated calves of G1 at any time points during the 27 day period after challenge. Viral genome was detected in 28.6% of the vaccinated calves of G2. In all the non vaccinated and challenged calves, viral genome was detected starting from D2 post infection (the mean Ct value of 28.13 was detected on the day of max viraemia, e.g. D9 post infection).

Conclusions

The Applicant provided evidence that the duration of immunity study of Zulvac® 8 Bovis vaccine in calves is ongoing. The interim results obtained to date are suitable in order to demonstrate that the duration of immunity is at least of seven months after the administration of a second dose of the vaccine (100% prevention of viraemia and vaccinated/challenged calves).

In absence of final results and in view of the preliminary information obtained on DoI the CVMP agreed to a DOI of 6 months which is reflected in SPC. The final report of study 115-B1-E-26-08 is awaited. The 12-month DoI results will be submitted.

Prevention of Transplacental Transmission

The Applicant confirmed that no data were available to date to show the efficacy of Zulvac 8 Bovis when used in pregnant animals.

Additional studies

No additional studies were reported (e.g. to support efficacy in other non-target ruminant species) rather than the dose/response study described above.

FIELD TRIALS

Data on field trials were not provided. This was acceptable as the CVMP Reflection Paper on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/ /IWP/105008/2007), stipulates that field trials may be omitted

OVERALL CONCLUSION ON EFFICACY

Satisfactory data were provided of the efficacy in the target species of vaccine preparations containing low antigen payloads and for the selection of the dose carried out in calves of the minimum age. Interim results supported duration of immunity of 6 months. No additional efficacy data were generated in pregnant animals of the target species.

Overall the CVMP concluded that the vaccine can be considered efficacious in the context of an authorisation of exceptional circumstances in the target species. In this respect the SPC reflects the current knowledge obtained by the submitted documentation.

5. BENEFIT-RISK BALANCE

Vaccination against BTV is a very important tool for the control of the disease and is also important for the 'safe' trade in live ruminants in accordance to OIE standards and EU legislation. In recognition of the urgent need to make suitable authorised products available the CVMP adopted a guideline regarding the minimum requirements for an authorisation under exceptional circumstances for vaccine for emergency use against BT (EMEA/CVMP/IWP/220193/2008). The benefit risk balance of the product has been based on the requirements of the above guideline and was considered favourable given the:

i) Epidemiological situation in Europe: Over the last ten years, the bluetongue situation in the EU has changed considerably with incursions of new serotypes, particularly in the last two years of serotype 8 into an area of the Community where outbreaks have never been reported before and which was not considered at risk of bluetongue. Furthermore, the onset of BTV-1 in northern Spain and south of France evolves by a spread of this serotype to the north with unknown consequences with regard to the epidemiology and pathology of a mixed infection with BTV-8. Co-infection by the two serotypes has been already notified in France. The recent observations of BTV-6 in The Netherlands and Toggenburg orbivirus in Switzerland add to the complexity of the epidemiological situation.

ii) Lack of authorised vaccines against BT: In this emergency situation, the concerned European member states have given temporary authorisations to various BTV-8 vaccines but so-far only one vaccine against serotype 8 has obtained a centralised authorisation.

iii) Sufficient quality of the product
-the production process respects the integrity of the viral particles and is robust, proving consistency of the manufactured batches, and thus insuring consistency of the forthcoming batches,
-each batch will be blended at a target antigen concentration based on its pre-inactivation titre
-each batch will be released on the basis of a reliable batch potency test in mice

iv) Sufficient safety of the product:
- sufficient data are available to exclude the presence of extraneous agents,
- the antigen is fully inactivated through a validated inactivation process,
- adjuvants and excipients used were already qualitatively and quantitatively used in other vaccines intended for ruminants,
- pharmacovigilance data already support safety of the vaccine under field conditions,

v) Sufficient efficacy of the product: the vaccine was shown to prevent viraemia caused by the bluetongue virus serotype 8.

No significant risks were identified when the product is used as indicated in SPC and under normal veterinary practice conditions. However the risk remains that the described benefits are based on limited information, which was submitted in the face of an emergency situation. On this basis the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that at present the overall benefit risk analysis is deemed positive and the quality, safety and efficacy of the product are sufficient to grant a community marketing authorisation under exceptional circumstances. However, the authorisation of the product will be subjected to annual re-assessment in order to recommend whether the authorisation should be continued or not. In addition, the commitments undertaken by the Applicant must be fulfilled, in order for the authorisation to revert to normal status, i.e. no longer exceptional and subject to annual review.