SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

BTVPUR Alsap 8 is an aluminium hydroxide ($Al(OH)_3$) /saponin adjuvanted vaccine intended for the active immunisation of sheep and cattle to prevent viraemia and to reduce clinical signs caused by the bluetongue virus serotype 8. The active substance of BTVPUR Alsap 8 is the inactivated bluetongue virus Serotype 8 (BTV 8).

The benefit of BTVPUR Alsap 8 is the stimulation of active immunity in sheep and cattle against the bluetongue virus, serotype 8. The vaccine dose is 1ml. The vaccination schedule consists of one injection given from 1 month of age, except in young animals born from vaccinated animals, in which case, vaccination should be delayed to 2.5 months of age. Primary vaccination of cattle includes a second injection of 1 ml dose given 1 month after the initial injection. Onset and duration of immunity are respectively 3 weeks after the primary vaccination course.

BTV 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. Acute form in sheep is usually characterised by pyrexia up to 42°, 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. Although BT 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 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. Recent 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).

2. QUALITY ASSESSMENT

COMPOSITION

The composition for a dose of 1 ml is provided in the following table.

Names of ingredients		Quantity per 1 ml dose	Function	Reference to standards
Active substance	BTV8 antigen	7.1 CCID50*	Induction of immunity	Merial
Adjuvant	Aluminum hydroxide	2.7 mg of Al^{3+}	Adjuvant	Merial
Adjuvant	Purified saponin	30 HU**	Adjuvant	Merial
Excipient and (Constituents of) Diluent	Silicone antifoam		Antifoam	Merial
	Phosphate buffer		Volume adjustment	Merial
	Glycine buffer			

* Equivalent to titre prior to inactivation (log10); ** Haemolytic units

CONTAINERS

The vaccine is presented in polypropylene bottles of 50ml containing 50 doses and 100ml containing 100 doses. They are packed in carton boxes of 1 or 10 bottles. The vaccine is also presented in a Type I glass bottle of 10ml containing 10 doses packaged in a carton box of 1 bottle. Each of the above three bottles (50ml, 100ml and 10ml) is sealed with a butyl rubber stopper and an aluminium cap.

DEVELOPMENT PHARMACEUTICS

The classical approach, was applied and can be described as follows:

- Define an active ingredient (AI) process (same process for all serotype) and validate it.
- Define a formulation (same formulation for all serotype) and validate it (using a well known formula: i.e. mix of aluminium hydroxide and saponin used for other similar vaccines)
- Set up the challenge model in sheep and cattle
- Define appropriate control tools

The development program for BTV vaccines in the early 2000s, followed a similar approach as the one used for the production of Foot and Mouth Disease (FMD) vaccines
The introduction of a purification step was chosen on the basis of the African Horse Sickness (AHSV) vaccine production. Removal of the majority of non particulate material including the inactivating agent (ethyleneimine) improved safety while allowing concentration of mainly viral particles. This removal was best achieved by the chromatography..

Choice of strain: The BTV 8 strain originated from an ill sheep during an outbreak in the Region of Ardennes, France in 2006. The identity of the strain was confirmed by RT-PCR on the Master Seed Virus. The history of the strain was provided. A Master Seed Virus (MSV) was constituted on BHKcells, and stored frozen Prior to vaccine production, the working seed virus (WSV) is expanded from the MSV into BHK cells and stored frozen.

Manufacturing process:
The vaccine antigen was produced in BHK cells; virus harvest was inactivated by a validated step process, then concentrated and purified by chromatography.
The chemical treatments and the processing of the viral harvest were considered appropriate as they improved the safety and efficacy profile of the vaccine by permitting an increase of the antigen content. In this way the antigen was purified and safety was improved .
The routine batches met the safety and efficacy standards as demonstrated in the submitted safety and efficacy studies. For the formulation of the vaccine classical adjuvants have been used such as aluminium hydroxide and saponin. They were selected as their safety and efficacy was demonstrated for similarly designed vaccines against FMDV which were produced by the Applicant. Because of the "exceptional circumstance" status of the application, the titre of virus (at harvest) just before inactivation was accepted as the critical parameter for the quantitative definition of the vaccine strength. At routine industrial production the vaccine is formulated to contain a defined amount of unconcentrated virus culture. To this aim, the limit of viral titre before inactivation was set at 7.1 $\log_{10}$ $CCID_{50}$/ml.
Taking into account that the consistency of production was demonstrated under the specific manufacturing process of the current vaccine, and considering that a challenge test in sheep will be performed as batch potency test, the titre before inactivation was considered acceptable by the CVMP under the exceptional circumstances of the present application.

Active ingredient-Analytical evaluation of the BTV antigen
The Applicant implemented different techniques to evaluate the robustness of the manufacturing process Overall on the basis of the submitted information it was evident that the process is leading to a purified enriched viral particle suspension, and that the production process was robust as only limited variability within different parameters could be seen.
Production of antigen
The Applicant clarified the production parameters of the active ingredient that are monitored during the process and the rationale for their choice.

Results from new analytical tools for the active ingredient process such as HPLC, PCR, Elisa VP7 were used to indicate the consistency of production.

Final product

Regarding the final product, a large number of batches were formulated with the method described earlier. The consistency was assessed through the final product testing performed on these batches

The parameters chosen to be monitored at this stage of the production were based on the assessment of the relevant results and are described below:

1 - Physical and chemical:

Regarding physical and chemical parameters, the results recorded show the consistency of the formulation through the compliance with specification from batch to batch.

2 - Safety:

All the results recorded show the consistency of the safety profile from batch to batch at twice a dose

3 - Efficacy:

Each batch produced is currently tested by challenge in sheep. As described earlier, serology in sheep cannot be used, as the BTV serotype 8 antigen in the final product does not consistently induces a measurable level of sero-neutralizing antibodies after one vaccine injection in sheep. The challenge results obtained effectively show that all the batches tested were fully protective in sheep.

A list of the control tests performed to the final product is presented below:

- Appearance
- pH
- Volume
- Free formaldehyde
- Quantification: Viral Content (titer before inactivation)
- Quantification: Antigen Content (ELISA)
- Potency in sheep
- Aluminium hydroxide
- Specific safety
- Bacterial and fungal sterility

Conclusions:

Taking into consideration the fact that:

- the production process was shown to be robust,
- the production process respected the integrity of the viral particles
- the viral content before inactivation will be of at least 7.1 $\log_{10}$ $CCID_{50}$/ml, while a lower titre was shown to still be efficacious in both sheep and cattle,
- each batch will be released on the basis of a challenge model on sheep, strengthened with regard to the previous proposed specifications in the initial file,

the CVMP concluded that sufficient guarantees are available on the analytical aspect for granting a marketing authorisation under exceptional circumstances.

Composition of the batches used in the clinical trials

The Applicant confirmed that all the experimental and production batches of the BTV active ingredient and finished product used in the safety and efficacy studies have been produced in the same manner. An exception has been the first experimental batches used in the early phases but they were found to be similar and in compliance with the current active ingredient production process description.

The CVMP concluded that the safety and efficacy studies were carried out with the appropriate antigen payload and the production batches used in the safety and efficacy studies were representative of those proposed for commercial batches.

METHOD OF MANUFACTURE

The production flow chart for the finished product (formulation, filling, and packaging) was provided. All stages of the manufacturing process were described in sufficient details. The virus is multiplied in growing cells. After the culture is stopped, and harvested, the culture is treated. An inactivation

process is carried out. The inactivated virus suspension is then concentrated and purified. Unless specified all the operations are conducted in closed circuits and all connections are sterilised by means which are in compliance with Eur. Ph. All calculations of the volumes of the different components were described in sufficient details. These constituents are sequentially added to antigen/buffer to obtain the final blend and then stored until filling**.**

Overall evidence was provided that primary packaging elements (bottles and closures) are sterilized by steam in compliance with the requirements of current Eur. Ph. Filling is carried out in clean atmosphere under laminar air flow of grade A located in an environment of grade B (EEC GMP classification). After closing, bottles are stored in a cold room at +2°C/+8°C for secondary packaging operations**.** All the bottles of vaccine coming from the same bulk and filled during the same cycle constitute a final lot. All the final lots prepared from the same bulk constitute a batch.

CONTROL DURING PRODUCTION

A flow chart detailing controls performed during production was provided**.** The following tests were described such as: Checking of the sterilising filter integrity, Monitoring of the sterilisation cycle, Temperature recording, Time recording,
For secondary packaging the controls are conventional ones such as checking of the filled volume, checking of the appearance of the product after capping, checking of the conformity (to a reference model) of the product presentation, etc.
Until dispatch, bottles of finished product are stored in a cold room (especially intended for the storage of finished products) at +2°C/+8°C.

CONTROL OF FINISHED PRODUCT

Finished products are checked for the parameters listed below. The methods, the frequency and pass criteria were provided.
A) General characteristics of the finished product: Appearance, pH, Volume, Free Formaldehyde
B) Identification and assay of AI: BTV8 component quantification by challenge in sheep
C) Identification and assay of adjuvants
D) Sterility and Purity tests
E) Safety tests

The Applicant provided the results of several batches of the finished product to show consistency of the quality from batch to batch. In addition, a full set of batch record data (including in- process and up-dated controls on the finished product) on three batches of finished product were provided.
On the basis of the information provided the CVMP concluded that the consistency of production was demonstrated and was adequately supported by the batch record data. The pooling of the antigen was clarified, and the concepts of batch, vaccine bulk and final lot were defined satisfactorily. The minimum / maximum size of standard production was also provided.

Validation studies
A summary of the studies carried out to validate different production processes of the finished product was presented.
Type of validation:
- Inactivation kinetics of BTV8 antigen
- Validation of active ingredient BTV8 inactivation control test
- Validation of titration of BTV8

The Applicant provided additional information in order to demonstrate that the production (small scale) process used for the experimental inactivation kinetics study was equivalent to the one used at industrial level for routine production. On the basis of the information provided the CVMP considered that the inactivation kinetics on a small scale production were satisfactory. The Applicant showed consistency of the test results.

Process validation

Regarding process validation, a large amount of data from several batches of active ingredients were provided to show the consistency of this process. Moreover, the same amount of information on several batches of finished product was also given to show the consistency of the vaccine quality from batch to batch. The consistency of the cell culture/virus system adopted for the manufacturing of the active ingredient was demonstrated. The consistency of the formulation of the finished product on a routine basis was also shown. All the BTV key active ingredients and finished products batches used in the trials were produced in the same manner whatever the size. In fact, the details of the production process of these key active ingredient batches showed that their production profiles are similar and in compliance with the current active ingredient production process description. Except from the batches used in the very first efficacy study with BTV2 the composition of the vaccine batches used in the key safety and efficacy studies complied with the current composition of the BTV vaccine. Thus, the safety and efficacy studies were carried out with the appropriate antigen quantity.
As a conclusion, the vaccine batches tested in the key safety and efficacy studies can be considered representative of the whole production process.

CONTROL OF STARTING MATERIALS

STARTING MATERIALS LISTED IN A PHARMACOPOEIA

Details were provided for the following substances, accompanied with a respective copy of the Eur. Ph. monograph and a certificate of analysis in order to show compliance of the tests performed:

Starting materials

- Calcium chloride dihydrate
- Disodium phoshate dihydrate
- Formaldehyde solution (35%)
- Magnesium chloride hexahydrate
- Potassium chloride
- Potassium dihydrogen phosphate
- Sodium chloride
- Sodium hydroxide
- Water for injection in bulk
- Polypropylene for containers for preparation for parenteral use
- Type-I glass bottles
- Butyl elastomer closure

The Applicant clarified that the absence of bovine and ovine extraneous agents in the MSV was shown in compliance with relevant EU legislation following testing for the following agents. In all cases the results were negative.

Agents to be tested for bovine species according to EU legislation

Adenoviruses, subgroups 1 and 2, Akabane virus, Aujeszky's disease virus, Bovine coronavirus, Bovine herpes viruses 1, 2, 4, Bovine leukemia virus (enzootic bovine leukosis), Bovine parvovirus, Bovine papular stomatitis virus, pseudocowpoxvirus, Bovine respiratory syncytial virus, Bovine viral diarrhoea virus, Brucella abortus, Cowpoxvirus, vaccinia virus, Foot and mouth disease virus, A, O, C ASIA1, SAT1, SAT2, SAT3, Lumpy skin disease virus, Mycobacterium tuberculosis and paratuberculosis, Mycoplasma SP., Parainfluenza 3 virus, Coxiella burnetti (Q-Fever), Rabies virus, Rift valley fever virus, Rinderpest virus, Vesicular stomatitis virus, indiana and new jersey.

Agents to be tested for ovine species according to EU legislation

Border disease virus, Borna disease virus, Brucella ovis, Chlamydia ovis, Looping ill virus, Nairobi sheep disease virus, Ross river virus, Brucella melitensis, Ecthyma contagiosum virus, Peste des petits ruminant virus.

STARTING MATERIALS NOT LISTED IN A PHARMACOPOEIA

Starting materials of biological origin

Information on the following starting materials of biological origin was presented

Starting material
BHK cells
BTV8 antigen
Bovine serum

Casein hydrolysate
Porcine trypsin
Purified saponin

BHK cells

BHK cell line is a baby hamster kidney cell line used as substrate for the production of BTV8 vaccine antigens.

Control and tests carried out on the MCB

In accordance with Eur. Ph. general text, and relevant EU documents, including the guideline on extraneous agents, samples taken from homogeneous batch of MCB or from passage levels are tested for general examination of fibroblastic appearance during amplification, and for

- Bacteria and fungal sterility: general and specific tests for *Brucella spp*. ; *Mycobacterium Coxiella Salmonella spp, Chlamydia spp*. and *Chlamydia abortus and Chlamydia pecorum*
- Mycoplasma sterility
- Extraneous agents: Absence of viral contamination was checked by using **general** (and **specific testing** (e.g. for viruses of **bovine** origin: **porcine** origin: **ovine/ caprine** origin: **different animal species origin**: Rabies virus (IF); **rodent species origin**. Relevant testing and results in accordance to extraneous viruses GL were reported
- Identification of species
- Karyology;

The range of passages allowed for production of virus goes up to MCB+20. .

The CVMP considered that the characteristics , including bacterial, mycoplasma and viral purity of BHK cell substrate used for the production of the BTV vaccine antigens are in general satisfactory. The testing conditions were relevant and acceptable.

BTV8 antigen

Origin and history: the virus strain was isolated from infectious material originated from an infected sheep during an outbreak of BTV. BTV8 serotype identity was confirmed by RT-PCR carried out on MSV. The treatment of the infectious material and the production of the BTV8 antigen were described in details, the production flow chart of BTV8 vaccine antigen was provided.

The Applicant clarified that the absence of bovine and ovine extraneous agents in the MSV was shown in compliance with relevant EU legislation following testing for the specific agents. In all cases the results were negative.

Preparation of active ingredient(s): The batches of the active ingredient are obtained from no more than five passages in BHK cells from MSV; virus is harvested, treated followed by clarification and centrifugation. Inactivation is carried out by addition of binary ethyleneimine (BEI). Final manufacturing stages include concentration, purification by chromatography. Each batch of active ingredient is tested for an infectivity titer (before inactivation), bacterial and fungal sterility.

Bovine serum

Assurance that the donor animals comply with the regulations concerning TSEs is supported by the provision of EDQM certificates of suitability. Purity tests and γ-irradiation are used as complementary measures to achieve a high security level against potential contamination.

The validation of the irradiation method was provided.

Casein hydrolysate

Casein hydrolysate is manufactured from hydrolysis of bovine casein made from bovine milk sourced from healthy animals (in compliance with EU legislation on TSE) declared fit for human consumption).

Controls for assessing viral purity and specific extraneous agents were described adequately.

Porcine trypsin

Porcine trypsin is manufactured from pancreas of swine that are declared fit for human consumption.

Controls for assessing viral purity and specific extraneous agents were described adequately

Purified saponin

Saponin is a liquid substance of vegetable origin. Controls were described adequately.

Starting materials of non-biological origin
Details of starting materials or components of non-biological origin, relevant preparations, control tests and certificates of analysis were provided. In this context the following substances were listed:

Starting material
Aluminium hydroxide°
Bromoethylamine Hydrobromide (BEA)
Chloroform
Glycine buffer
Hydrocloric acid
PBS
Stabiliser F2
Silicon antifoam

In House preparation of media
Description of components methods of preparation, (including the sterilisation procedure), basic controls carried out during preparation have been provided to support the quality of the following media prepared in house. The information provided reassurance that the in-house preparation and quality of the following media is satisfactory:

Starting material
GMEM
Virus Maintainance Medium (VMM)

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies
The 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, and 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.
Starting materials falling within the scope of TSE assessment used in the manufacturing process and presenting no risk of transmission of TSE are: BHK cells, BTV8 antigen, casein hydrolysate, bovine serum. Relevant certificates of suitability or where appropriate risk assessment where provided.

Overall a satisfactory assessment of the above components was conducted in order to demonstrate that the risk of transmission of TSE is minimised by the documented and recorded sourcing of materials, by the nature of the animal tissues used in manufacturing (of low or no detectable infectivity), by the production processes, and by a series of minimising factors which lower the risk if any. It was found that the starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

STABILITY

No studies were conducted to support the stability of BTVPUR Alsap 8. The Applicant justified the absence of stability data by the fact that production of BTVPUR AlSap 8 started in 2008. However, studies were provided in order to show an overview of the stability of BTV vaccines. Data were presented on the stability of one production batch of the monovalent BTV2 vaccine and BTV4 vaccines and of one production batch of the bivalent BTV2 & 4 vaccine. Overall the conclusions from this study were taken into account by the CVMP but were considered of limited value to the scope of this application.

The CVMP concluded that in line with the provisions of the document EMEA/CVMP/IWP/105008/2007, a maximum interim stability of 12 months can be assigned.

The Applicant confirmed that the same protocol will be implemented on three batches of each of the 50ml, polypropylene bottle-presentation and 10 ml, glass bottle-presentation. Stability results obtained from three batches of 100ml polypropylene bottlr presentation of the vaccine were provided and were considered acceptable.

OVERALL CONCLUSIONS ON QUALITY

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

- a batch with a low antigen content was shown efficacious in both sheep and cattle,
- the production process allows production of consistent batches, with now strengthened specifications on both "titre before inactivation" and "specifications of the challenge test on sheep",

the CVMP has sufficient guarantees to assume that forthcoming batches will be efficacious in both sheep and 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 both species).
All these assurances are considered sufficient for granting a marketing authorisation under exceptional circumstances, but not for a full marketing authorisation, for which the whole production process needs to be revisited, and for which a reliable, immunologically significant and validated potency test is requested.

3. SAFETY ASSESSMENT

Introduction and general requirements
BTVPUR ALSAP 8 is a conventionally produced, liquid and ready-to-use, BEI inactivated vaccine, adjuvanted by aluminium hydroxide ($Al(OH)_3$) and purified saponin. One-ml dose is recommended to be administered by subcutaneous route in sheep and cattle. The vaccination schedule consists of one injection given from 1 month of age, except in young animals born from vaccinated animals, in which case, vaccination is delayed to 2.5 months of age. Primary vaccination of cattle includes a second injection of 1 ml dose given 1 month after the initial injection. According to current European legislation, 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 has also to be carried out. In addition, according to Annex I Part 7 of Directive 2001/82/EC, "*The dose to be used shall be that quantity of the product to be recommended for use and containing the maximum titre or potency for which the application is submitted*". However, in this specific respect, in light of 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) representative experimental batches or standard production batches can be used. The same reflection paper, allows also 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, are used to fulfil safety requirements. According to the same paper, field trials are not required as long as proof of evidence of the safe use of BTV vaccines containing different BTV serotypes is provided under laboratory conditions. No additional data was provided in order to support the safety of the vaccine in animals of non-target ruminant species. The Applicant presented limited results specifically to demonstrate the safety of BTVPUR AlSap 8 vaccine but made reference to a series of laboratory studies carried out in sheep and cattle with batches of vaccines formulated with different BTV serotypes antigen which had a similar composition in adjuvants/excipients.

A. SAFETY ASSESSMENT

LABORATORY TESTS

Preliminary work included supportive antigen dose studies carried out using experimental and production batches of monovalent (BTV2 or BTV4) and of experimental bivalent BTV2&4 vaccine preparations. Safety conclusions were extrapolated from a series of trials performed with different payload of BTV9 vaccine antigen combined with BTV2/4 vaccine antigens.
The preliminary results of two studies aimed to provide some information on the safety of experimental BTV8 vaccine preparations were also presented.

Following the request of the CVMP the Applicant presented results from studies with animals of the minimum age recommended for vaccination, as well as data was generated in breeding animals.

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:

- Observation parameters
- Clinical signs after vaccination
- Impact on body temperature (T°)
- Local reactions (LR)
- Impact on growth performance
- Post mortem examination (including selected investigation of injection site)
- BTV serotype specific SNT

Safety of the administration of one dose/Safety of an administration of an overdose/Safety of the repeated administration of one dose

Safety of the administration of one dose
A number of one dose studies performed with different inactivated BTV vaccines but of similar composition in regards to excipients and adjuvants were presented in this part of the dossier in order to support the safety profile of BTVPUR Alsap 8.

i) Assessment of safety and efficacy of six BTV2 experimental vaccines in sheep by vaccination and challenge.
The objective was to evaluate the safety of the subcutaneous injection of 1ml/dose of six experimental vaccine prototypes containing different BTV2 antigen and adjuvants payloads.
Systemic and local reactions observed in this study were, in general, acceptable. However, the data are of supportive nature only due to limited information on the batches of the vaccines used.

ii) Assessment of safety and efficacy of six BTV4,BTV2&4 and BTV2 vaccines in sheep by vaccination and BTV4 challenge.
The objective was to evaluate the safety of the subcutaneous injection of 1ml/dose of inactivated experimental BTV4 vaccines, formulated with different amounts of antigen in comparison with an experimental inactivated bivalent BTV2&4 vaccine, a production batch of a monovalent BTV2 inactivated vaccine (from the same manufacturer).
The systemic and local reactions observed were acceptable. The data however, are of supportive nature only due to limited information on the batches of the vaccines used.

iii) Assessment of safety and efficacy, by vaccination and challenge, of vaccines formulated with different BTV9 antigen payloads.
The objective was to evaluate the safety (and the efficacy) of the subcutaneous injection of 1ml/dose of five experimental vaccine preparations formulated with different payloads of BTV9 antigen with/without standard amount of BTV2&4 antigens in comparison with an experimental bivalent BTV2&4 vaccine preparation. The results were satisfactory. Data generated from the experimental vaccine preparations used in this study, were taken into account to support the safety of BTVPUR AlSap 8.

iv) Assessment of safety and efficacy of four monovalent BTV4 vaccines formulated with antigen batches produced with different processes.

The objective was to evaluate the safety of the subcutaneous injection of 1ml/dose of BTV4 experimental vaccine preparations formulated with different payloads of pilot antigen batches that were produced with two different processes.
Local and general reactions recorded in this study were, in general, compatible with an acceptable level of safety of the vaccine preparations.

v) Assessment of safety and immunogenicity of a BTV2 experimental vaccine in bovines.
The objective was to evaluate the safety of an inactivated experimental BTV2 monovalent vaccine in cattle. Local and general reactions observed in this study are, in general, acceptable.

Safety of an administration of an overdose/ Safety of the administration of the vaccine at minimum age

A number of studies were presented under this section by the Applicant. Some the overdose studies were conducted in animals of minimum age and therefore this part of the safety assessment is also presented here. Most of the studies were conducted with BTV vaccines of different serotypes but of similar composition regarding adjuvants and excipients. For two studies which were conducted with serotype 8, the Applicant provided only preliminary data in the format of interim reports. In total five studies are presented under this section and are detailed below:

Calves:
Safety assessment of a bivalent inactivated BTV-4/BTV-8 (Bluetongue virus serotypes 4 and 8) vaccine containing high antigen payloads in young calves of minimum age. (including a double dose) This an intermediate report which provided only preliminary safety data collected from all animals after each vaccination. Results of histopathological examination of the injection sites were missing and are expected to be presented in the final report. The study was designed to assess the safety in young calves of a bivalent BTV-4/BTV-8 vaccine formulated at high antigens payloads after administration of a double dose (overdose study) on D0 and single doses on D14 and D28 repeated single doses administration study).

On the day before the start of the study (D-1), calves from 23 to 30 days old, seronegative to BTV, were randomly allocated (according to their bodyweight on D-1) to 2 groups (G1 and G2) as described in the table below.

Group	Treatment		
	D0	**D14**	**D28**
G1	Vaccine high Ag content 2 mL	Vaccine high Ag content1 mL	Vaccine high Ag content 1 mL
G2	Physiological saline 2 mL	Physiological saline 1 mL	Physiological saline 1 mL

° the vaccine was formulated with the equivalent of 8 mL of non concentrated BTV-4 and of 8 mL of non concentrated BTV-8 antigens per 1-mL dose

Follow up
The vaccine was administered by the subcutaneous route. The safety of the vaccine with high antigen content (double dose) was assessed through monitoring the rectal temperature, general condition (apathy, anorexia, polypnea, salivation and tremor), local reactions and bodyweight gain. The examination of local reactions included macroscopic examination and palpation. The presence of swelling, redness, necrosis and/or pain was recorded at injection site; in case a local reaction was observed, the length and width of the reaction was measured. This was carried out daily starting from the day of each injection of the vaccine, 4 hours later, up to D4 after each injection and weekly respectively from D7 to D49.

Results

- Rectal temperature: a moderate and transient increase of rectal T° was observed following the administration of repeated dose of the vaccine. A statistically significant difference was observed only after the 2nd administration of the vaccine
- General condition: rare and transient apathy or decrease of appetite one day after vaccination was observed
- Local reactions:
  - limited swelling reactions appearing 1-2 days after the administration of an overdose on D0 and resolving 4 weeks after vaccination was recorded.
  - moderate swelling reactions occurring 1 day after repeated administration of the vaccine on D14 and D28 and resolving almost completely within 21 days were observed
  - granulomas of very limited size (max volume 2.5 cm3) were only observed in vaccinated calves on 3-4 injection sites at each vaccination session
- Body weight: no impact on bodyweight gain.

Conclusions:
This study provided evidence to support the general safety profile of the vaccine under application in calves of minimum age.

Lambs:

- ***Safety assessment of a commercial BTVPUR AlSap 8 (Bluetongue virus serotype 8) vaccine in a flock of suckling lambs.***

The study was aimed to assess local and general tolerance in young lambs of a commercial batch of the vaccine under application. The trial was conducted in suckling lambs from a conventional flock, in June 08. Conventional suckling lambs, aged between 14 and 74 days at the beginning of the trial, free from ELISA BTV antibodies, were randomly allocated to two groups, one consisted controls and another of vaccinates. A commercial batch of BTVPUR Alsap 8, was used for vaccinates and the placebo was a physiological saline solution. On D0, lambs from the vaccinated groups were administered 1 ml of the vaccine, whereas the lambs from the control group were administered physiological saline at the same volume and route.
The safety of the vaccine was assessed through individual monitoring of the temperature, general and local reactions) of controls and vaccinated animals. Clinical examination was performed the day of the injection, daily during the 4 days following the injection, and then on the 7th and the 14th day after the injection. Clinical monitoring included measuring of the rectal temperature, recording of any abnormal clinical sign observed, and assessing the size of local reactions at injection site. All pathologies and/or mortality observed, all the treatments administered were recorded, all dead animals underwent a necropsy.

Results

- A moderate and transient rise in rectal temperatures was observed in both vaccinated and control groups following the administration of test treatments. The differences between groups were not statistically significant. The maximal individual increase recorded up to 7 days following the administration of the test treatments, was 1.2°C and 1.3°C for the vaccinated and control groups, respectively.
- No visible local reactions were recorded.
- Diarrhoea or soft stools were the only general signs observed in several lambs from both groups, which were associated to heavy worm infestations, evidenced by coproscopy and necropsies.

Conclusions
The CVMP considered that the overall safety profile of the commercial batch of the vaccine under application was demonstrated in this study.
The safety related sections of two interim reports from two studies conducted with the BTVPUR Alsap 8 vaccine which were mentioned earlier are presented below. The studies were mainly focused on demonstrating the efficacy of the current vaccine and therefore they are described in details in Part IV.

a) Assessment of safety and efficacy, by vaccination and challenge in sheep, of vaccines formulated with different BTV-8 antigen payloads (including a double dose)

As far as safety is concerned, the objective of the first study was to assess the safety for 3-4 month old sheep of 6 experimental BTV8 vaccine preparations formulated with different payloads of BTV8 antigens in the presence or absence of BTV2 and/or BTV4 antigens. All the experimental preparations contained 2.7 mg of aluminium hydroxide and 30 HU of saponin per dose. Six groups (from A to F) of 5 sheep were once vaccinated with 1ml /dose of each experimental vaccine preparations according to the following scheme:

Group n.	Payload of antigen		
	BTV-2	BTV-4	BTV-8
A	0	0	0.1x
B	0	0	0.25x
C	0	0	1x
D	1x	1x	2x
E	1x	1x	1x
F	1x	1x	0

A seventh group (g. G) was enrolled including 6 sheep which were left as untreated controls for both safety and efficacy testing. For safety assessment rectal T° was measured in all sheep on D-4, D-3, D0 (before vaccination), D0+4h, and then daily from D1 to D4. Clinical monitoring for general reactions (apathy, anorexia, polypnea, ptyalism and tremor) and clinical examination (by measurement with a calliper) was scheduled on all sheep at D0 and D0+4h, then daily from D1 to D4, and thereafter on D7 and D14 after vaccination. No further details of the study were provided with the exception of the general conclusions that stated the following: 1) max T° increase was not significantly higher in the vaccinated groups (including group D) as compared to the controls; 2) very limited local reactions were observed immediately after the vaccination in all vaccinated groups, 3) no significant general reaction was observed after vaccination.

b) Assessment of the efficacy of an inactivated BTV-8 vaccine administered in two injections to bovines, assessed by a virulent BTV-8 challenge

The safety of a BTV8 vaccine preparation containing 0.25x vaccine antigen/dose and adjuvanted with 2.7 mg of aluminium hydroxide and 30 HU of saponin per dose was also investigated in cattle. Each animal of group (A) was vaccinated on D0 with the vaccine preparation under test. Group (B) animals remained untreated and served as control. To assess the safety of the vaccine, individual rectal T° was recorded; general reactions were monitored; injection sites were inspected for local reactions. Monitoring took place after 1st and 2nd vaccination. No individual data or statistical evaluation of data was presented. Limited reactions (max size of 12 and 24 cm^2 lasting for 14 days) in two vaccinated animals was observed. General tolerance to the vaccine was concluded from the absence of either significant difference between the increase of rectal T° recorded in vaccinated and control animals and from the absence of general reactions in none of the two groups.

Conclusions:

Overall the CVMP considered the results satisfactory as the final reports were provided during the first annual re-assessment..

Another study was also presented in this part of the dossier, using a similar vaccine containing a different BT serotype.

Safety assessment of an overdose administration of a monovalent BTV4 vaccine to sheep.

Objective: To evaluate the safety of an overdose administration of a monovalent BTV4 vaccine in sheep. Animals were randomised in 2 groups (vaccinated and controls).

Administration route and vaccine scheme

On D0, a 3 ml dose was administered subcutaneously to each sheep of the vaccinated group. Controls were left untreated.

Results

Clinical observation: no systemic reaction was reported in controls whereas sporadic (on D3 and D4) cough was recorded in one vaccinated sheep.
Rectal T°: in the two groups, maximal average increase of rectal T° was recorded on D0+4h (the highest individual/the average values were 40.7°C/39.9°C and 40.2°C/39.8°C in vaccinated and controls respectively). Differences were not statistically significant.
Local reactions: reactions at injection site were only recorded in all vaccinate sheep. The largest reactions (from 1-12 cm^2, average 5.1) were recorded 14 days after administration of the vaccine.

Conclusions
The overdose administration of the vaccine did not result in any significant general reaction. The conclusions were found acceptable.

Safety of the repeated administration of one dose
The studies presented in this part of the dossier used similar vaccines containing different BT serotypes, thus allowing the extrapolation of safety data to the vaccine under application. Some of them also included an overdose investigation.

i) Assessment of the safety of an overdose and repeated doses of a bivalent BTV2&4 vaccine formulated with a high antigen payload, in less than 3-month-old-calves.
Objective
To evaluate the safety of an overdose and of a repeated doses administration of an experimental batch of BTV2&4 vaccine in calves.
Calves were randomly allocated in 2 groups (vaccinated and controls respectively).
A BTV2&4 vaccine was used which contained.2x antigen/dose of each of BTV2 and BTV4 vaccine antigen, 2.7 mg/ml aluminium hydroxide and 30 HU/ml.
The placebo was a physiological saline solution.

Administration route and vaccine scheme
On D0, D14 and D28 each calf from the vaccinated group received by subcutaneous route, a dose of the vaccine preparation as follows.
1st vaccination (D0): 2ml (double dose) of the vaccine;
2nd vaccination (D14): 1ml (1 dose);
3rd vaccination (D28): 1ml (1 dose);
Control calves were treated with placebo solution on the same days, with the same dosage, route and site as vaccinated animals.

Results
Clinical observation: with the exception of one control calf, no further general reaction was ever reported in animals of both groups.
Rectal T°: in the two groups, an increase of rectal T° was recorded already on D0+4h following each vaccination. The average values were respectively 39.3, 39,8 and 39.1°C with an individual peak of 40.8°C recorded after second vaccination in vaccinated animals and 39.3, 39.5 and 39.2 in controls with an individual peak of 40.4°C recorded after second vaccination.
Differences were reported as statistically significant for max increase of rectal T° in vaccinated groups (compared to controls) after 1st and 2nd vaccination, while a tendency to significance was reported after the third one.
Local reactions: reactions at injection site were recorded in all vaccinated calves. Local reactions rapidly emerged after each administration of the vaccine, reaching max size one day after. Average/range reaction size (in cm^2) values one day after injection were the highest after the 3rd vaccination.
39.5/27-58 after 3rd vaccination; (global max reaction size: 39.5/27-58)
Local reactions were frequently associated to an enlargement of the pre-scapular draining lymph nodes.

Conclusions
The overdose administration of the vaccine did not result in any general reaction and had no impact on growth performance. On average, pyrexia was higher following overdose vaccination than after

subsequent single dose vaccination; however pyrexia was considered acceptable. Local reactions were considered acceptable.

ii) Safety assessment of a BTV2&4 vaccine formulated with a high antigen payload, following administration of repeated doses to 3-month-old-sheep.

Objective
To evaluate the safety of an overdose and of a repeated (over)dose administration of an experimental batch of BTV2&4 vaccine in sheep.
Administration route and vaccine scheme
Sheep were randomly allocated in two groups (vaccinates and controls).
A BTV 2+4 vaccine was used which contained per 1 ml dose: 2x of BTV2 and BTV4 and 2.7 mg/ml aluminium hydroxide.

Results/Conclusions:
The results of this study showed that there was abnormal clinical reaction following repeated administration of the vaccine (3 vaccinations) and there was no impact on the growth performance. Transient and moderate hypothermia was observed and lesion at the injection site of acceptable level.

Assessment of safety and immunogenicity of a BTV2&4 inactivated vaccine in young cattle following repeated administration.

Objective
To evaluate the safety of repeated administration of one dose of a production batch of bivalent BTV2&4 vaccine in cattle. The study was provided only as supportive information for the safety assessment of BTVPUR AlSap 8.

Calves from 2 to 2.5 month old were allocated in 3 groups. Group 1 and 2 were vaccinates and Group 3 controls. A production batch of the BTV2&4 vaccine was used and physiological saline was used as placebo for the controls. On D0, and subsequently, on D28, each calf received the assigned treatment (1 ml of vaccine or placebo as appropriate for the group) by subcutaneous route on the right (D0) and left (D28) lateral face of the neck.

Results
Clinical observation: No systemic reaction was reported in vaccinated or control calves.
Rectal T°: There was no statistical difference between vaccinates and controls following two first and second vaccination.
Local reactions: Following the first vaccination 80% of vaccinated calves presented local reactions. More extensive reactions were recorded 1-2 days after administration of the vaccine. The size of the reactions ranged from 1 to 28 cm^2. After 2-3 weeks, 1 cm^2 local reaction was still present in 20% of calves. Following the second vaccination 60% of vaccinated calves presented local reactions. Still, no local reaction was present in the 20% of calves for which no local reaction was reported after first vaccination. No local reaction was detected in the 20% of the calves which presented the maximum local reactions after first vaccination. More extensive reactions were recorded 1-2 days after administration of the vaccine. Size of reactions ranged from 1 to 12 cm^2. After 2-3 weeks, 1 cm^2 local reaction was still present in 30% of calves.
Conclusions:
The CVMP considered that the local and general reactions observed in this study were acceptable. However, concerns were raised on the measurement of the local reactions.

Examination of reproductive performance

1) Safety of a bivalent BTV2/BTV4 vaccine with high antigens payload in pregnant ewes.
This study was designed in order to assess the safety in pregnant ewes of a bivalent experimental batch of BTV2 and BTV4 vaccine formulated with high antigens payloads. The vaccine was administered to pregnant ewes during either the first or second half of gestation.

Study design

Four days before the starting of the trial (D-4), primiparous or multiparous ewes, at approximately 7 or 18 weeks of pregnancy (at vaccination), were randomly allocated to 4 treatment groups described in the following table.

Group	Treatment	Pregnancy stage
A	BTV2/BTV4 vaccine on D0	Approximately 7 weeks
B	Placebo on D0	Approximately 7 weeks
C	BTV2/BTV4 vaccine on D77	Approximately 18 weeks
D	Placebo on D77	Approximately 18 weeks

On D0, animals of group A received a 1ml injection of the vaccine under test, containing a high payload of BTV2/BTV4 antigens (2x BTV-2 and 2x BTV-4 antigens per 1-mL dose), and amounts of adjuvants as the ones used to formulate the vaccine under application. The vaccine was administered by subcutaneous route. Physiological saline was administered to control animals of group B. The same procedure was followed on D77 for animals from groups C (vaccinates) and D (controls).

Follow up

Clinical monitoring included recording any abnormal clinical sign observed and rectal temperatures. Animals were monitored on a daily basis from D0 (before vaccination) to D4 for groups A and B; from D77 (before vaccination) to D81 for groups C and D. In addition, the impact of vaccination on the reproductive performance was specifically investigated, by registering, for each ewe, the number of aborted lambs, and at lambing, the number of born alive and dead born lambs. Growth of lambs was monitored, starting immediately after birth, and on the day of weaning.

Results

Four abortions occurred (1 in the control group, 2 in ewes of group C, one in one ewe of group D), of which 2 associated with the death of the ewes. The retrospective analysis of the farm conditions excluded that the deaths could be attributed to vaccination. Sporadically, a moderate increase of rectal T° was recorded in vaccinated animals (up to a max of 1.5°C in one vaccinated animal of group C). No statistically significant difference was observed between groups for rectal temperatures (T°).

The total and mean numbers of born alive and dead lambs observed in each treatment group were similar.

No statistically significant difference was observed between groups. A descriptive analysis was performed of necropsy lesions and suspected causes of death of lambs between birth and weaning. The total number of weaned lambs and the mean total weight of weaned lamb per ewe observed in each treatment group were similar. The statistical analysis confirmed that the total weights of weaned lamb per ewe were not significantly different between groups. The Relative Average Daily Weight Gain (RADWG) of the lambs observed in each treatment group was similar. There was no statistically significant difference between groups.

Conclusions

Overall, the Applicant's conclusions that a satisfactory safety profile of the vaccine under test was demonstrated in pregnant ewes vaccinated during the first and second stage of pregnancy are sustainable. Therefore, extrapolation of the results obtained from this study to the current application can be accepted.

2) Safety of a bivalent BTV-4/BTV-8 vaccine with high antigens payload in pregnant cows. – (Field trial)

Study design:

Safety data were reported for this study in an interim report initially and later in a final one. The study was designed in order to assess the safety in pregnant cows of a bivalent BTV-4/BTV-8 vaccine formulated at high antigens payloads after two administrations at 4 weeks of interval. A total of 96 conventional, 3-9 years old cows and heifers, at different stage of pregnancy and SN antibody negative to BTV4 and BTV8, were enrolled in the study. The animals were assigned to two treatments groups (G0, including placebo treated controls, and G1, including vaccinated cows). The randomization process took into account the location, age category and increasing number of gestation. On the date

of inclusion (D0) and four weeks later (D28), each cow in G1 received by subcutaneous route, 1 ml injection of an experimental batch of vaccine BTV4/BTV8, which was formulated with the equivalent of 2x BTV-4 and 2x BTV-8 antigens per 1-mL dose . Each cow in G0 received physiological saline the same way as in G0.

Follow up:
The safety of the vaccine was assessed through a daily clinical monitoring of each animal (rectal temperature, general reactions, apathy, anorexia, polypnea, salivation and tremor) for four days after each vaccination. The assessment of the reproductive performance was also conducted. This was carried out by monitoring of any abnormality occurring during pregnancy and at calving and therefore the number of born alive, dead, aborted calves was recorded.

Results:
From the preliminary results of this safety study it could be concluded that a bivalent BTV-4/BTV-8 vaccine formulated at high antigens payload:

- Only moderate and non specific clinical signs were recorded.
- A limited and transient temperature increase was included. Only a small number of vaccinated and control animals exhibited a max temperature increase above 1°C.
- The total number of calving and abortions observed in each treatment group were similar and three cases of abortion were recorded, one among vaccinated cows and two among controls.

Conclusions:
The CVMP considered that results provided supportive evidence, to the general safety profile of the vaccine under application in pregnant animals at different stages of pregnancy.

Examination of immunological functions
No specific study was carried out.
There is no reason for suspecting impairment of the immune system due to the vaccination.

Interactions
Since 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 besides the one conducted in pregnant cows were not provided. The Applicant referred to the safe use of BTV4 and/or BTV2&4 in Corsica, Spain, Portugal and Italy. Evidence of the safe use of BTV vaccine preparations in sheep and cattle was given by relevant literature which was 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 presented a Periodic Safety Report (PSUR) based on the use of the product in the different vaccination campaigns in Member States. The information included in the report was considered satisfactory.

Environmental Risk Assessment
Phase 1 assessment was carried out, providing evidence that there is no potential risk for the global environment. No phase 2 assessment was deemed necessary.
No hazard is expected to the environment in light of the nature of the vaccine, in particular of the antigen (inactivated) and adjuvant(s) (pharmacologically inert substances). Additionally, no special concern is posed by the final product taking into account the safety of packaging, the limited number of injections, the maximum quantity administered to animals, the route and method of administration, and disposal. Therefore the level of risk is minimal justifying the absence of phase 2 assessment.

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 in order to justify the absence of any specific study of residues being conducted.
The Applicant's approach is acceptable for the CVMP.

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	All	EC 2796/95
Saponin	All	EC 1433/96

Withdrawal period
Zero days.

OVERALL CONCLUSIONS ON SAFETY

The provision of additional safety data demonstrated the safety of the vaccine in sheep and cattle of minimum age and in pregnant animals of both target species. Additional safety data were also provided for goats. Overall, the safety profile of BTVPUR Alsap 8 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
BTVPUR Alsap 8 is indicated for the active immunisation of sheep and cattle to prevent infection, viraemia and clinical signs caused by the bluetongue virus serotype 8.
The vaccination schedule consists of one injection given from 1 month of age, except in young animals born from vaccinated animals, in which case, vaccination should be delayed to 2.5 months of age. Primary vaccination of cattle includes a second injection of 1 ml dose given 1 month after the initial injection. Onset and duration of immunity are claimed respectively in 3 weeks after primary vaccination course. Duration of immunity has not been fully established yet, however, revaccination is recommended as one yearly injection preferably before peak vector season in the epidemic areas. According to Annex I Part 8 of Directive 2001/82/EC, and EP 2005 general monograph, Vaccina ad usum veterinarium, (evaluation of efficacy of veterinary vaccines), *tests shall be carried out during the development of the vaccine to demonstrate the efficacy when administered by the recommended route and method of vaccination and using the recommended schedule to each species and category for which use of the vaccine is recommended. The dose to be used shall be that quantity of the product to be recommended for use and containing the minimum titre or potency (expected at the end of the period of validity*) *for which the application is submitted*. However, based on the recommendations provided 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) either representative experimental batches or standard production batches can be used. No additional data was generated to demonstrate the efficacy in animals of non-target ruminant species. Field trials were not strictly required for this type of application, nor did the Applicant provide any data related to them.

LABORATORY TRIALS

A series of studies carried out using Merial vaccines with different BTV serotypes were presented.
In addition, the preliminary results of two (safety and) efficacy studies carried out in sheep and in cattle using an experimental batch of BTVPUR AlSap 8 vaccine were presented. Upon request of the CVMP the Applicant presented additional studies with the BTV8 serotype to support the use of the vaccine in young animals of the minimum age and to support the duration of immunity.

Establishment of a Challenge Model
The characteristics and relevance to the epidemiological situation, of the virus inoculum which was used in laboratory studies was discussed by the Applicant. The biological properties of the different field and the laboratory adapted strains are different. In sheep, reproduction of clinical signs with some serotypes and isolates may be difficult. The mortality rate and the severity of clinical signs can vary depending breed, the age of the animals, the strain of the virus and the interaction with the environment. Bovines although susceptible to BTV develop mainly a subclinical infection and a long lasting viraemia. Therefore viraemia represents the only parameter normally to be tested under experimental conditions for this species. However, during recent BTV8 epidemics in NW Europe visible lesions of the nasal mucosa, salivation, fever, conjunctivitis dysphagia, nasal discharge, depression, congestions of the oral mucosa and lameness were also reported in infected cattle.

No specific study (either in sheep or in cattle) was carried out in order to validate a challenge model using BTV8 serotype. However, reference was made to a series of validation studies in which the effect of factors like virus dose, type of inoculum, route of inoculation, passages in cell cultures, were evaluated in order to establish a valid challenge model for BTV 2 and 4 serotypes. Diagnostic techniques in the above studies included Seroneutralisation (SNT), ELISA, RT-PCR and virus isolation. Details of these techniques were provided.

The use of qRT-PCR alone for detection of viraemia was noted in several studies, including those in which BTV 8 challenge was performed. The Applicant presented a qualitative and quantitative validation study of the qRT-PCR used to detect and quantify the viraemic status of the animals. The technical characteristics of the test were described in details. The validation of the technique included demonstration of specificity, linearity, limit of quantification, precision and sensitivity with the limit of detection. This latter was validated at 3.14 log10 copy number/ml. A comparative evaluation between infection titers and qRT-PCR results was also carried out.

On the basis of the provided information the CVMP concluded that the qRT-PCR used for the detection of viraemia in the efficacy studies was qualitatively and quantitatively validated. Its relevance either as diagnostic tool or for the quantitative significance of positive values in terms of the potential for transmission of infectious virus was satisfactorily demonstrated.

Efficacy in calves of minimal age :
The Applicant provided the results of an additional efficacy study carried out in calves of minimal age, whereas bibliographic data were provided in order to support the extrapolation of the results to 1 month old lambs, born to non immune ewes. The CVMP concluded that for a full authorisation, efficacy data obtained from sheep of minimum age will be required, however for an authorisation under exceptional circumstances the principle of extrapolation can be accepted.

Efficacy in young calves of an inactivated BTV-8 vaccine against a BTV-8 challenge. Merial, 2008.
Objective: The objective of the study was to assess the protection induced in young calves (one month old, or less) by an experimental vaccine preparation, containing a low antigen payload, against a virulent BTV-8 challenge carried out 22 days after the second vaccination (three weeks interval). A certificate of analysis was provided for the experimental vaccine batch used for this study. The vaccine contained low amount of active ingredient BTV8 in 1 ml dose of each vaccine and the amount of adjuvants as per the proposed SPC.

Male calves from 23 to 30 day old healthy and seronegative to BTV, were randomly allocated in 2 groups as follows: Group 1: calves were vaccinated with 1 m dose of the test vaccine by the subcutaneous route, (D0), and 3 weeks later (D21). Group 2: calves were left as untreated controls.
Twenty-two days after the last vaccination (D43) all calves were submitted to a virulent challenge with BTV-8. Following the challenge the efficacy of the vaccine was assessed through monitoring of clinical signs attributable to BTV infection and the general condition of the animals. Clinical monitoring included the measurements of rectal temperature (T°). Blood samples for the detection of viraemia were collected on the day of challenge (D43), then, starting five days later (D48), at different time points during 4 weeks after challenge (D71). The presence of specific SN antibody to BTV8 was investigated on D0 (at 1st vaccination), D21 (at 2nd vaccination), D35 (14 days after 2nd vaccination), D43 (at challenge), D71 (4 weeks after challenge).

Results:
After challenge (and excluding a calf with intercurrent digestive disorder at vaccination time which subsequently succumbed to BTV), there was a statistically a significant difference in the maximum temperature recorded between the vaccinated and the control group.

A Global Clinical Score (GCS) was calculated. A statistically significant difference in GCS was demonstrated between the vaccinated and the control group.

Regarding viraemia the Applicant considered that when no viral genome was detected the titre was equal to the limit of detection of 3.14 log10 RNA copies/ml. This value was established through an adequate process of validation of the qRT-PCR. The amount of RNA found in each sample, was expressed in Log10 number of RNA copies per millilitre of blood.

Five days after challenge (D48), in 80% of control animals viral RNA was detected. From 7 days after challenge until the end of the study, viraemia was always detected in control animals. With the exception of one vaccinate calf (which was detected positive from D48 up to its death) in any other vaccinated animals viral RNA was not detected at any time point during the monitoring period. There was a statistically significant difference in AUC between the vaccinated and the control group.
Controls remained sero-negative to BTV-8 until challenge. In the vaccinated group, no increase in the BTV-8 antibody titres was observed after the 1st vaccination, and only a weak response was recorded after the 2nd vaccination (D21). 40% of vaccinated animals remained negative on D35 and 20% of calves were still negative at the time of challenge (D43). The serological monitoring provided evidence for the absence of correlation between serological response and protection.

Conclusions:
Based on the results of this study, and with the exclusion of one vaccinate calf, the Applicant concluded that the administration of two doses of the experimental vaccine of BTVPUR Alsap 8 with a low antigen payload resulted in a significant protection of calves vaccinated at a minimum age, against specific BTV clinical signs and total protection against viraemia. The CVMP considered that the Applicant's conclusions on the efficacy of the experimental batch of vaccine used in this study were sustainable. The study clearly allowed the evaluation of the challenge conditions and the assessment of viraemia (through a validated test method).
The intermediate reports of 2 more efficacy studies conducted with BTVPUR Alsap 8 were also submitted and are presented below:

A) Assessment of safety and efficacy, by vaccination and challenge, in sheep of vaccines formulated with different BTV8 antigen payloads

Objective: To evaluate (the safety and) the efficacy of the subcutaneous injection of 1ml/dose of six experimental vaccine preparations formulated with different payloads of BTV8 antigen with/without standard amount of BTV2 and 4

Study design
Sheep of 3-4 month old were randomly allocated to 7 groups. Six groups (A to F) were vaccinated and 1 group (G) of five animals was the control group. random allocation was not described.

Six experimental vaccines with different payloads of BTV8 antigen were used. Some of them contained additionally BTV2 and 4 antigens. All of them contained the same amount of 2.7 mg of aluminium hydroxide and 30 HU of saponin per dose. Each vaccine preparation was allocated to groups as per the following table.

Group	Payload of antigen		
	BTV2	BTV4	BTV8
A	____	____	0.1x
B	____	____	0.25x
C	____	____	1x
D	1x	1x	2x
E	1x	1x	1x
F	1x	1x	0

Sheep in group G acted as control for the challenge experiment. These animals remained untreated.

Administration route and vaccine scheme
On D0, each sheep (with the exception of controls) was subcutaneously injected - with 1ml/dose of respective vaccine preparation.

Post-vaccination follow-up (efficacy)
Blood samples for SNT against BTV2, 4 and 8 were collected from all sheep on D0, D14, D21, D31 (before challenge) and then on D45.

Challenge
The challenge inoculum was a virulent BTV8 field isolate. Animals were challenged 31 days after vaccination (D31) with 3 ml of the challenge material (red blood cells from infected sheep). Animals were monitored for clinical signs observed during BTV infection (by daily and global clinical scoring-D/G-CS), including measurement of the T° starting from 5 days post challenge (from D36) and thereafter, daily until D45. Blood samples for detection of viraemia by qRT-PCR were collected. 5 days after challenge (D36) and on, D38, D40, D43 and D45.

Results
Clinical monitoring: Maximum peaks of hyperthermia were recorded seven and six days after challenge respectively in control animals and in 60% of sheep from the group which was not vaccinated with BTV8 antigen. The increase of rectal T° was reported to be of no significance in any BTV8 vaccinated sheep. Clinical signs were reported to have occurred mostly in controls. Viraemia was detected by qRT-PCR as soon as 5 days after challenge in all controls and in 40% of sheep from group F. All these sheep were found positive at all dates of analysis. Viraemia was never detected in any other animals.
SNT antibodies: On D0, all animals were seronegative to BTV 8 antigen. Control sheep of group G and animals of group F remained seronegative until challenge.
Seroconversion to BTV8 was generally evident on D21 followed by a further increase in all (but group F) groups on D31 (before challenge). A similar anamnestic response to challenge virus was recorded on D45 in all control and vaccinated sheep.

Conclusions
Study results demonstrated complete and significant protection of the animals which were vaccinated with BTV8 vaccines against a BTV8 challenge. This was related to the prevention of viraemia in animals, even for those vaccinated with a vaccine containing a low BTV8 antigen payload.

B) Efficacy of an inactivated BTV8 vaccine administered in two injections to bovines, assessed by a virulent BTV8 challenge
Objective: To evaluate (the safety and) the efficacy conferred in bovines by 2 injections of an experimental vaccine preparation containing BTV8 antigen

Study design

Adult bovines, free of BTV8 antibodies, were randomly allocated to two (A and B) groups. Animals of group A were vaccinates whereas animals of group B were left as untreated controls. The vaccine used contained 0.25ml of BTV8 antigen and 2.7 mg of aluminium hydroxide and 30 HU of saponin per dose. No placebo was used in control animals.
On D0 and D28 each bovine of group A was subcutaneously injected with the vaccine.
Blood samples for SNT against BTV8 were collected from all bovines on D0, D14, D28, D42, D51 (before challenge) and then on D79.

Challenge: The challenge was carried out using a virulent BTV8 field isolate. Animals were inoculated 23 days after the second vaccination (D51) with 3 ml of the virulent challenge material Animals were monitored for increase in rectal T° and major clinical BTV signs starting on the day of challenge and then daily for 28 days after challenge. Blood samples for detection of viraemia by qRT-PCR were collected on D51 (before challenge), 5 days after challenge (D56), and then on D58, D63, D65, D67, D70, D72, D74, D77 and D79.

Results

Clinical monitoring:

Maximum peaks of hyperthermia in control animals were recorded eight days after challenge whereas no increase of rectal T° was observed among animals in the vaccinated group. Clinical signs were reported to have occurred mostly in controls. Viraemia was detected by qRT-PCR as soon as 5 days after challenge (D56) in 60% of controls
By day 58 also the remaining controls were found positive. Thereafter, all controls were found positive at all dates of analysis. Max peak of viraemia detection was recorded 9 days after challenge. On average max viraemia was 7.7 ±0.2. Viraemia was never detected in any of the vaccinated animals.
On D0, all animals were seronegative to BTV 8 antigen. Control bovines of group B remained seronegative until challenge. Seroconversion to BTV8 was only evident 14-23 days after the second vaccination. A significant increase of BTV8 SNTs was recorded in control animals after challenge, whereas only a slight increase of SNTs was recorded in vaccinated bovines after challenge.

Conclusions

Despite the limited number of animals, study results demonstrated a significant seroconversion to BTV8 among BTV8 vaccinated animals; a complete and significant clinical protection of the BTV8 vaccinated animals related with a complete and significant prevention of viraemia,

Supportive efficacy studies

Eleven studies using vaccines which contained different BTV serotypes than BTVPUR Alsap 8 were presented in support of the efficacy claims. The results of these studies were considered as supportive but not pivotal in relation to efficacy. Some of them were also presented in the safety part as they contained relevant information about safety.

1) Evaluation of the safety and efficacy of prototype vaccines against Bluetongue serotype 2 virus by sero-neutralisation and challenge

Objective: To evaluate the efficacy of the subcutaneous injection of one or two doses (given in 1 or in 1.5 ml) of five prototype vaccines (A, B, D, E and F) containing different BTV2 antigen payloads starting. Efficacy was demonstrated by serological (seroneutralization) follow up and by challenge of vaccinated sheep, in comparison to the efficacy of a live attenuated vaccine

Study design: Sheep were randomly distributed in 9 groups.
Five BTV2 inactivated, prototype vaccines (A, B, D, E, F) produced at pilot scale were used and one BTV2 live one. Physiological saline solution (PSS) at 1 ml/dose was used as placebo in pure controls animals (C).

Post-vaccination follow-up

Blood samples for detecting virus neutralizing antibody to BTV2 were collected on D-7, before each vaccination and every week after each injection until the end of the study

Challenge

The challenge consisted of red blood cells collected at the peak of the viraemia from two sheep. Animals were inoculated via the intradermal route with the virulent challenge. Challenge was carried out 4 weeks after the first vaccination injection.

Post-challenge follow-up

Clinical observation: rectal temperature, general behaviour (good, apathy, depression, prostration) and clinical signs were recorded for 3 consecutive days prior to challenge and on frequent intervals post challenge.

Viremia: blood samples were collected on the day of challenge (D42), daily from D44 to D52, and then on D56, D63and D70 post-challenge.

Results

All control animals presented severe changes of their general behaviour and hyperthermia. The individual/average T° was 41.9/41.1±0.7°C and was recorded 7 days after challenge. Specific signs of BTV were recorded between 6-8 and 7-12 days after challenge, respectively. Viraemia was detected as soon as 2 days (D44) after challenge in 60% of the sheep. Peak of viraemia was demonstrated on 5 days after challenge (D48). The highest viral titers recorded were until D52 (max average 4.70 ELD_{50}/ml).

In general, sheep of all vaccinated groups remained healthy without major changes in their body conditions or abnormal increases of rectal T°.

Sporadic signs of BTV were recorded in few vaccinated sheep. With the exception of one sheep in group 7 none of the vaccinated sheep showed viraemia throughout the entire period of observation.

Antibody titres remained unchanged in control animals until challenge. All vaccinated groups showed an increase of SNT after one injection, whereas the second injection had a booster. After challenge animals of control group seroconverted showing the highest individual (4.20) and average (3.85 ±0.34) SNTs to BTV ever recorded throughout the study. In vaccinated animals there was no evident anamnestic response to the virus challenge.

Conclusions

The efficacy against a severe BTV2 challenge was demonstrated for all prototype vaccines similarly to a modified live vaccine currently used to combat epizootics of BTV2 in Europe.

The CVMP considered that the above conclusions are sustainable although the results of this study can only be considered within the general context of the preliminary work aimed to building confidence in vaccines against BTV.

2) Assessment of safety and efficacy of six BTV2 experimental vaccines in sheep by vaccination and challenge

Objective: To evaluate the efficacy of the subcutaneous injection of 1ml/dose of six experimental vaccine prototypes containing different BTV2 antigen and adjuvants payloads

Results: All vaccinated sheep were protected against hyperthermia, clinical signs and viraemia. In this study lack of correlation between SNTs and protection was clearly demonstrated. BTV ELISA antibody can not be used for assessment of vaccination/protection.

Conclusions

The CVMP noted the results of this study. Although a number of issues still need to be addressed the CVMP supports the conclusion that in this study the lack of correlation between SNTs and protection was clearly demonstrated.

3) Assessment of safety and immunogenicity of a BTV2 experimental vaccines in bovines

Objective: To evaluate the immunogenicity of an inactivated experimental BTV2 monovalent vaccine by vaccination on D0 and D21 of cattle, and testing for BTV2 SNT and ELISA BTV antibody.

Results: At the end of the study, the vaccine induced BTV2 SNT comparable to those induced by the same vaccine in sheep and that were found protective against a virulent homologous challenge. BTV ELISA antibodies were detectable after the second administration of the vaccine preparation.

Conclusions

The CVMP considered that as it was not demonstrated that SNT did correlate with protection, protection against a homologous challenge can not be automatically predicted or extrapolated based on SNT.

4) Assessment of immunogenicity and protection provided by a bivalent BTV2&4 vaccine against a BTV2 or BTV4 challenge.

Objective: To evaluate the immunogenicity and the protection provided by a production batch of the current vaccine in sheep

Results**:** All vaccinated sheep were protected against hyperthermia, clinical signs and viraemia. Post challenge serological results indicated a complete absence of cross reactivity between BTV2 and BTV4.

Conclusions

In general, the CVMP considered that the Applicant's conclusions on the outcome of this study can be supported. As a consequence of the experimental conditions of this study, onset of immunity in sheep should be set at 4 weeks following the completion of the primary course of vaccination in this target animal species.

5) Immunogenicity of a BTV2&4 bivalent vaccine in bovines

Objective: To evaluate the immunogenicity of a production batch of bivalent BTV2&4 vaccine by measuring SNT vs BTV2 and BTV4 in cattle.

Results**:** Administration to cows of two-1ml doses of the vaccine at 28 days interval induces the production of BTV2 & 4 virus neutralizing antibodies. Individual SNT after second vaccination are suggestive of a possible viral protection.

Conclusions

The CVMP concluded that the uptake of the vaccine was demonstrated. As correlation however between SNT and protection has not been established, no extrapolation regarding viral protection can be made.

6) Assessment of safety and immunogenicity of a BTV2&4 inactivated vaccine in young cattle

Objective: To evaluate the serological response following primary course vaccination of calves with a production batch of bivalent BTV2&4.

Conclusions

On the basis of the results the CVMP concluded that the immunogenicity of the test vaccine was demonstrated

7) Assessment of safety and efficacy of six BTV4, BTV2&4 and BTV2 vaccines in sheep by vaccination and BTV4 challenge

Objective: To evaluate the efficacy against a moderately virulent BTV4 challenge of the subcutaneous injection of 1ml/dose of three inactivated experimental BTV4 vaccines formulated with different amounts of antigen in comparison with an experimental inactivated bivalent BTV2&4 vaccine, a production batch of a monovalent BTV2 inactivated vaccine (from the same manufacturer) and a commercially available BTV4 attenuated vaccine

Study design

Sheep were randomly allocated in 7 groups. 6 of those groups were vaccinates and 1 was controls.

Three inactivated experimental BTV4 vaccines, formulated with different amounts of antigen were used (0.5x, 1x, 2x of antigen per dose respectively); one inactivated bivalent BTV2&4 vaccine; one monovalent BTV2 inactivated; and a BTV4 attenuated vaccine. Placebo was not used.

On D0, each sheep in a specific group was subcutaneously injected with 1ml/dose of the corresponding vaccine

Post-vaccination follow-up

The vaccine was well tolerated by sheep; blood samples for detecting virus neutralizing antibody to BTV2 and BTV4 were collected at different time points after vaccination

Challenge

The challenge inoculum consisted of red blood cells collected from one infected sheep and was given via the intradermal route. On the day of challenge (D29), sheep were challenged with 1 ml of the inoculum.

Post-challenge follow-up

Clinical observation: after challenge, sheep were monitored for rectal temperature, general behaviour and conditions and for specific signs of BTV
Serology: blood samples for SNTs against BTV2 and BTV4 and BTV ELISA antibody were collected at different time points before/after challenge.
Viraemia: blood samples were collected for detection of viraemia by RT-PCR and 5, 7, 9, 12 and 14 after challenge.

Results
Clinical monitoring:
Clinical signs following challenge were globally moderate in controls and mostly observed in those animals. Viraemia was detected by RT-PCR as soon as 5 days after challenge in 80% of control sheep and 80% sheep of group 5. Viraemia was never detected in any other vaccinated sheep. SNTs to BTV2 remained unchanged in control animals until challenge, thereafter seroconversion was recorded. BTV2 vaccinated sheep had no BTV4 SNTs, whereas in BTV4 vaccinated sheep, homologous seroconversion was observed as early as at D7, SNTs resulting globally higher in sheep vaccinated with a live modified BTV4 vaccine. With the exception of sheep of the attenuated vaccine, a clear anamnestic response to challenge was observed in all vaccinated animals to levels of SNTs similar or even higher than those recorded in controls animals after challenge.

Conclusions
Administration of vaccines formulated with 4 and 8 ml of BTV4 vaccine antigen resulted in a clinical protection and in a complete prevention of detectable viraemia (RT-PCR) in sheep challenged with a virulent BTV4 inoculum. Vaccine formulated to lower amount of vaccine antigen provided partial clinical protection but totally prevented viraemia. In BTV vaccinated sheep, neither clinical nor virological protection was obtained. As a result of this study, it was concluded that the kit used to measure BTV ELISA antibody can not be used for assessment of vaccination.
The CVMP considered that the overall conclusions from this study can only be taken into account in the general context of preliminary work aimed to build confidence in vaccines against BTV.

8) Assessment of safety and efficacy of four monovalent BTV4 vaccines formulated with antigen batches produced with different processes
Objective: To evaluate the efficacy of BTV4 experimental vaccine preparations formulated with different payloads of pilot antigen batches that were produced with different processes.
Results
Administration of test vaccines resulted in a strong clinical protection associated to a nearly complete prevention of detectable viraemia (RT-PCR) even in sheep vaccinated with a vaccine formulated with the lowest antigen payloads. Efficacy was demonstrated whatever the process.
Conclusions
Overall conclusions from this study can be taken into account to support the modifications introduced to the initial manufacturing process of BTV vaccines.

9) Assessment of efficacy of a bivalent BTV2&4 inactivated vaccine by vaccination and challenge in cattle
Objective: To evaluate the efficacy of a production batch of bivalent BTV2&4 vaccine by vaccination/challenge against BTV2&4 in cattle
Study design
4 to 5 month old calves from a BTV-free herd were enrolled in two groups, one to be vaccinated and one to be used as controls. Fourteen days before challenge vaccinated and control groups were further divided into two subgroups, thus finally allocating the animals to 4 groups identified as Controls BTV2 (g.1)/Controls BTV4 (g.2)/Vaccinates BTV2(g.3)/Vaccinates BTV4 (g.4). The potency of the vaccine used was provided with the amount of aluminium hydroxide (2.5 mg/ml) contained in each dose.On D0 and D 28 animals of groups g.3 and g.4 were injected subcutaneously with a 1 ml/dose of the vaccine.

Challenge

Challenge was carried out on 37 days after 2nd vaccination with a virulent BTV2 or BTV4 field isolates. Cattle of each respective group were challenged by subcutaneous route with 1 ml of a viral suspension of BTV2 or BTV4.

Results

Vaccination resulted in the prevention of detectable viraemia which would statistically signify a virological protection of at least 83.8% (95% confidence interval) of vaccinated animals.

Conclusions

The CVMP considered that the overall conclusions from this study can only be taken into account in the general context of preliminary work aimed to build confidence in vaccines against BTV.

10) Assessment of safety and efficacy, by vaccination and challenge, of vaccines formulated with different BTV9 antigen payloads (The safety part of this study is discussed in Part III of the CVMP AR)

Objective: To evaluate the safety (and the efficacy) of the subcutaneous injection of 1ml/dose of five experimental vaccine preparations formulated with different payloads of BTV9 antigen with/without standard amount of BTV2&4 antigens in comparison with an experimental bivalent BTV2&4 vaccine preparation.

Study design: **S**heep were randomly allocated into 6 groups of vaccinates and 1 group of controls. Six experimental vaccines containing the same amount of 2.7 mg of aluminium hydroxide and 30 HU of saponin per dose and varying amounts of BTV antigens were used. Each vaccine preparation was allocated to a group as presented on the table.

Group	Payload of antigen (eq ml)		
	BTV2	BTV4	BTV9
A	____	____	0.1x
B	____	____	0.25
C	____	____	1x
D	1x	1x	1x
E	1x	1x	2x
F	1x	1x	0

Some sheep were allocated in group G and acted as controls for the challenge experiment. These animals remained untreated.

On D0, each sheep in a specific group (was subcutaneously injected-with 1ml/dose of the corresponding vaccine.

Challenge

Challenge with 3ml of a virulent BTV9 field isolate was carried out 23 days after vaccination (D23). Animals were monitored daily for clinical signs including measurement of rectal temperature. Blood samples for detection of viraemia by qRT-PCR were collected on 5 days after challenge and at different time points thereafter.

Results

Study results demonstrated a significant protection of the animals vaccinated with BTV9 containing vaccines against BTV9 challenge, associated with a significant prevention of viraemia, even at the lowest BTV9 antigen payload.

Conclusions

The CVMP considered that although the results are noted they can not be used to support the efficacy of the current vaccine. Protection induced by specific BTV serotype vaccines is indeed recognized to be restricted to the homologous serotype challenge strain. However, from the outcome of the present study, it is evident the lack of correlation between antigen payload and clinical protection (including increased body T°), between antigen payload and SNTs, between SNTs and protection.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

No specific study was performed to investigate the impact on vaccination of pre-existing maternally derived antibodies (MDAs) to vaccine antigen. The Applicant has provided a review of existing documents and data which would likely support the evidence that the persistence of MDAs in lambs and calves (as a consequence of either natural infection or vaccination of ewes and heifers) can be for

2 to 3 months. Therefore, cattle and sheep may be efficiently vaccinated from 2.5 months of age. Overall, based on the current knowledge in such a specific and not totally clarified field, the considerations expressed by the Applicant are sustainable to the aim of a marketing authorization given under exceptional circumstances. The Applicant should be aware that data from specific experimental/field trials should be provided to obtaining a full marketing authorization. In the absence of studies regarding the impact of MDA on vaccination, appropriate warnings should be included in the relevant section of SPC. The Applicant has committed to provide further information on the impact of MDAs.

Duration of Immunity

Some supportive evidence for one year duration of immunity was provided in a study using 5 month old sheep with a commercial batch of BTVPUR AlSap 2 vaccine. The results demonstrated significant clinical and complete virological protections 1 year after a single administration of the vaccine**.**

Regarding BTVPUR AlSap-8, the Applicant provided only information on a vaccination/challenge trial carried out in sheep 6 months after vaccination. An interim report of such a study was provided:

Duration of immunity of an inactivated BTV-8 vaccine administered to sheep in one or two injections- Protection conferred by the vaccine against a virulent BTV-8 challenge performed 6 and 12 months after vaccination.

The objective of this study was to assess the duration of immunity after one or two administrations in sheep of an experimental batch of a monovalent BTV8 vaccine with a low antigen load. The protection was only evaluated in a group of vaccinated animals submitted to a BTV-8 challenge which took place 6 and 12 months after vaccination.

On day 0 of the study (D0), male sheep, of 5.7-6.2 months of age, seronegative to BTV were randomly allocated in 5 groups (G0a, G1a, G1b, G2a and G2b) of vaccinates and 1 group (G0b) of controls.

- In group G1a and G1b, sheep received a 1mL dose of experimental vaccine preparation under test by the subcutaneous route.
- In group G2a and G2b, sheep received 2 doses of the vaccine under test at a 28-day interval.
- In the two control groups (G0a and G0b) sheep remained untreated.

The experimental design of the study is summarized in the following table.

Group	Sub-group	Administration of the vaccine		BTV-8 challenge	
		D0	D28	6 months after vaccination	12 months after vaccination
G0	G0a	---	---	yes	---
	G0b	---	---	---	yes
G1	G1a	yes	---	yes	---
	G1b	yes	---	---	yes
G2	G2a	yes	yes	yes	---
	G2b	yes	yes	---	yes

Animals were submitted to a daily clinical monitoring after each vaccination, in order to check general health conditions, respiratory and digestive disorders, locomotion impairment. Serological monitoring using a serum neutralization test was also performed on a regular basis between week 0 (W0) of the study and W49. Six months post-vaccination (Day197), the animals from groups G0a (controls), G1a (one vaccination regimen applied) and G2a (two vaccinations regimen applied) were challenged. Efficacy of the two vaccination regimens was assessed through clinical monitoring including the recording of increase of rectal temperature, monitoring of general body condition, Appearance of clinical signs attributable to BTV infection, and viraemia. Sampling for the detection of viraemia was carried out from five days after challenge on D202, during 14 days after challenge. Serological response (SN) was also measured 14 days after challenge (e.g. on D211).

Results were submitted to statistical analysis using the same parameters set for other relevant efficacy studies.

The experimental challenge was successful in controls, as all these animals showed clinical signs of BTV infection and detection of viraemia. Post-challenge observations demonstrated that both vaccination regimens (1 or two vaccinations) were fully protective against a BTV-8 challenge performed 6 months after vaccination (W28). This conclusion was supported by a significant reduction of hyperthermia and clinical signs, in comparison to controls and complete protection against viraemia (measured by validated qRT-PCR).

Some data became available on duration of immunity in cattle from the study below:

Efficacy of an inactivated BTV-8 (Bluetongue virus serotype 8) vaccine against BTV-8 challenge, performed on calves, 6 months after completion of vaccination The objective of the study was to assess the protection afforded by an inactivated BTV-8 vaccine containing a low antigen payload, 6 months after completion of basic vaccination scheme. Some information regarding the Duration of Immunity in cattle was provided by this study but due to the very low antigen content tested the Applicant has decided to stop this study and to start a new using a vaccine batch with an antigen content at least similar to the current minimum antigen content. The new study will take place between November 2009 and February 2011. A final report is expected for 2Q2011.

FIELD TRIALS

Data on field trials were not provided. This was accepted by the CVMP in light of the current requirements described 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) which state that field trials may be omitted.

OVERALL CONCLUSIONS 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. The Applicant provided the results of an additional efficacy study carried out in calves of the minimum age, whereas bibliographic data were provided in order to support the extrapolation of the vaccine's efficacy to 1 month old lambs, born to non immune ewes, of immunological behaviour of 4 month old lambs. No additional efficacy data were generated in pregnant animals of each target species. This circumstance is reflected in the SPC.

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

V. RISK-BENEFIT 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. The benefit risk balance of the product has been based on the requirements of the above guideline and was considered favourable for the product given the:

i) Epidemiological situation in Europe: Over the last ten years, the Bluetongue situation in the EU has considerably changed 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 regards 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 there no BT vaccine has obtained a full 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 ensuring consistency of the future batches,
-the viral content before inactivation will be of at least 7.1 $\log_{10}$ $CCID_{50}$/ml, whereas a titre 10 times lower was shown efficacious in both sheep and cattle,
-each batch will be released on the basis of a strengthened challenge model on sheep,

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 supported safety of the vaccine under field conditions
- each batch will be released on the basis of a strengthened challenge model on sheep,

v) Sufficient efficacy of the product: the vaccine was shown to prevent viraemia (based on the detection limit set at 3.14 log10 copy number/ml, such a value indicating that no virus transmission occurs by midge biting) and reduce clinical signs 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 subject to annual re-assessment in order to recommend whether the authorisation should be continued or not. In addition, satisfactory answers must be given to all other concerns, in order for the authorisation to revert to normal status i.e. no longer exceptional and subject to annual review.

The CVMP, having reviewed the evidence of compliance with the specific obligations as submitted by the Marketing Authorisation Holder by the time of the first annual re-assessment and having re-assessed the benefit/risk profile of the veterinary medicinal product, as stated recommended the continuation of the Community Marketing Authorisation for the veterinary medicinal product under exceptional circumstances.