EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines and Product Data Management

Scientific discussion

This module reflects the initial scientific discussion for the approval of BTVPUR AlSap 2-4 (as published in November 2010). For information on changes after this date please refer to module 8.

1. Summary of the dossier

BTVPUR AlSap 2-4 is an aluminium hydroxide saponin adjuvanted vaccine intended for the active immunisation of sheep to prevent viraemia and to reduce clinical signs caused by bluetongue virus serotypes 2 and 4. The active substance of BTVPUR AlSap 2-4 is the inactivated bluetongue virus Serotype 2 and 4 (BTV2 and BTV4).

The benefit of BTVPUR AlSap 2-4 is the stimulation of active immunity in sheep against the bluetongue virus, serotypes 2 and 4. 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. Onset and duration of immunity correspond to 3 and 5 weeks after the primary vaccination course for serotype 4 and serotype 2, respectively.

Bluetongue Virus (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. The 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. Serotypes 2 and 4 have been responsible for outbreaks in regions of East and South Europe (e.g. BTV2 and 4, in Corsica, in Spain, Portugal and in Italy; BTV4 in Greece).

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

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2. Quality assessment

Composition

The composition for one dose of 1 ml is provided in the following table:

Names of ingredients		Quantity per 1 ml dose	Function	Reference to standards
Active substance	BTV2 antigen	6.8 – 9.5 CCID50*	Induction of immunity	Merial
Active substance	BTV4 antigen	≥ 7.1 CCID50* ≤ 8.5 CCID50*	Induction of immunity	Merial
Adjuvant	Aluminium hydroxide	2.7 mg of Al^{3+}	Adjuvant	Merial
Adjuvant	Purified saponin	30 HU	Adjuvant	Merial
Excipients	Silicone antifoam		Antifoam	Merial
	Phosphate buffer		Volume adjustment	Merial
	Glycine buffer			

* equivalent to titre prior to inactivation (log_{10})

Container

The vaccine is filled in 100 ml (100 doses) and 50 ml (50 doses) polypropylene bottles (Ph. Eur. 3.1.6) and 10ml (10 doses) Type I glass bottles respectively closed with a chlorobutyl (type I) rubber stopper (Ph. Eur. 3.2.9) and sealed with an aluminium cap. Satisfactory information on the quality standards, including certificates of analysis for the bottles and rubber closures were provided.

Development Pharmaceutics

The management of BTV vaccines as underlined in the Guideline for the minimum requirements for an authorisation under exceptional circumstances for vaccines for emergency use against bluetongue EMEA/CVMP/IWP/220193/2008 was also consistent with the approach taken for the avian influenza vaccines and within the frame of the multi-strain dossier concept. As a result the vaccine production processes were standard besides the selected vaccine strain(s) the production of the Master Seed Virus (MSV), the amount of the active ingredient (AI) and adjuvants/dose. This allows the easy replacement of different serotypes without changing the quality of final products, thus ensuring, the quick availability of the vaccines when needed. The same principle, on which the field epidemiology of Foot and Mouth Disease (FMD) management was based, was also adopted for the selection of BTV strains. In addition, the vaccine production benefited from the experience already gained by the applicant from an already authorised Bluetongue serotype 8 vaccine and from another vector borne disease vaccine, such as African Horse Sickness, from the use of classic starting materials of standard quality and finally from further refinement of the production process which limited the use of starting materials of animal origin.

Choice of strains:
The seed BTV2 and BTV4 strains originated from outbreaks of BTV in sheep in Corsica and were selected for optimal antigen supply for an inactivated vaccine. The history of each strain was provided. For each serotype, a Master Seed Virus (MSV) was constituted and stored frozen. Control tests ensured the virus stock was free from potentially contaminating bacteria, fungi and mycoplasma. The identity of the seed virus serotype was confirmed by serotype specific RT-PCR. . Prior to the vaccine production, the working seed virus (WSV) is expanded from the MSV stock into BHK cells and stored frozen.

Manufacturing process:
The vaccine antigens are produced in BHK cells; each virus harvest is inactivated by a validated step process, then concentrated and purified by chromatography.

The chemical treatments and the processing of the viral harvests were considered appropriate as they improved the safety and efficacy profile of the vaccine by permitting an increase of the antigen content. 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 FMD which were produced by the applicant.

Establishment of minimum protective titre:

The titer of virus (harvest) just before inactivation was the critical parameter selected for the quantitative definition of each serotype. The product was formulated based on a defined amount of virus culture per dose. To this aim, a minimum limit of viral titre before inactivation of 6.8 and 7.1 $\log_{10}$ CCID*50/ml was set for serotype 2 and serotype 4, respectively.

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.

* infective dose for 50% of the cell cultures

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 processes such as HPLC, PCR, ELISA were used to indicate the consistency of production. As a conclusion, the consistency of the whole antigen process was demonstrated by all parameters measured or the analytical tools used. The CVMP thus concluded that the consistency of the formulation of BTVPUR AlSap 2-4 vaccine can be ensured on a routine basis.

Final product:
Regarding the final product, several 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:

Physical and chemical:

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

Safety:

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

Efficacy:

Each batch produced is currently tested by challenge in sheep. The serology in sheep cannot be used, as the BTV serotypes 2 and 4 antigen in the final product do not consistently induce a measurable level of sero-neutralizing antibodies after one vaccine injection. The challenge results obtained 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 6.8 and 7.1 l $\log_{10}$ CCID50/ml for serotype 2 and 4, respectively,
- each batch will be released on the basis of a challenge model on sheep,

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 data provided 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 was 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.

Evidence was provided that primary packaging elements (bottles and closures) are sterilized 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 (according to the EC 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: 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 tests on the 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: BTV2 and 4 components 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 batch records data. 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 for each serotype:
- Validation of the titration
- Inactivation kinetics
- Validation of inactivation control test
- Process validation

Regarding process validation, a large amount of data from several batches of active ingredients was provided to show the consistency of this process. Moreover, 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 copy of the relevant Eur. Ph. monograph and a certificate of analysis that show conformity of the test performed by the applicant:

Starting material
Calcium chloride dihydrate
Disodium phoshate dihydrate
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 for containers
Butyl elastomer closure

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

The information on the following starting materials was presented:

Starting material
BHK cells
BTV2 antigen
BTV4 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 both BTV2 and BTV 4 vaccine antigens.

Control and tests carried out on the Master Cell Bank (MCB):

In accordance with Eur. Ph. general text, and relevant EU documents, including Notice for Guidance (NfG) on extraneous agents, samples taken from homogeneous batch of MCB or from passage levels were tested for general examination of fibroblastic appearance during amplification, and for:

- Bacteria and fungal sterility
- Mycoplasma sterility
- Extraneous agents
- Identification of species carried out on MCB and MCB+20
- Karyology on MCB and MCB+20.

The range of passages allowed for production of virus goes up to MCB+20 (20 passages). The same controls as for MCB are carried out on WCB with the exception of the identification of species and karyology. The CVMP considered that the characteristics, including bacterial, mycoplasma and viral purity of BHK cell substrate used for production of BTV vaccine antigens were in general satisfactory. The testing conditions were relevant and acceptable.

BTV2 antigen
Origin and history: the virus strain was isolated from an infected lamb during an outbreak of BTV2. The BTV2 serotype was confirmed by RT-PCR

BTV4 antigen
Origin and history: the virus strain was isolated from an infected lamb during an outbreak of BTV4. BTV4 serotype was confirmed by RT-PCR.

The treatment of the infectious material for both strains and the production of both antigen were described in details, as the production flow chart of BTV2-4 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.

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
Bovine blood serum from adult, calf and donor animals is used as component of cell culture medium. Assurance that the donor animals comply with the regulations concerning TSEs was given by the provision of EDQM certificates of suitability.

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

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

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 for the following substances:

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

In House preparation of media

Description of constituents, method of preparation, including sterilisation procedure carried out according to the requirements of current Eur. Ph., basic controls carried out during preparation were provided to support the quality of the following culture media:

-Glasgow's modified Eagle's medium (GMEM)

-Virus Maintenance Medium

The information provided reassurance that the in-house preparation and quality of the media is satisfactory.

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

The assessment of 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. An assessment of the risk was provided that covered the starting materials included below, that falls within the scope of TSE assessment either as material of ruminant origin or because of the manufacturing process.

Starting materials:

Active Ingredient: BTV2 antigen, BTV4 antigen
BHK21 cells
Casein hydrolysate
Adult bovine serum
Calf serum and donor calf serum
From those the following raw materials of biological origin are involved in the manufacturing process:

Raw materials containing materials from ruminant origin:

Bovine serum

Bovine milk products*:* Casein hydrolysate

An assessment was conducted in order to demonstrate that the risk of transmission of TSE is significantly minimised by the documented and recorded sourcing of animals (animal-derived material of known and controlled origin), by the nature of animal tissues used in manufacturing (low or no

detectable infectivity), by the production processes, and by the negligible risk posed as a series factors would likely lower the risk if any, such as high dilution of the materials used, route of administration and maximum/minimum number of dosage injected. Adequate certifications of suitability or conformity of the materials used were provided as appropriate.

The CVMP concluded 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

Data were presented on the stability of one production batch of the monovalent BTV2 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/220193/2008, 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 100 and 50ml, polypropylene bottle-presentation and 10 ml, glass bottle-presentation.

Overall conclusion on quality

All the data and clarification provided by the applicant can be considered sufficient for granting a marketing authorisation under exceptional circumstances, when taking into account the risk-benefit balance for BTV serotype 2 and 4, 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 sheep,
- 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”,

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

All these assurance are considered sufficient for granting a marketing authorisation under exceptional circumstances.

3. Safety assessment and residues

Introduction and general requirements

BTVPUR AlSap 2-4 is a conventionally produced, liquid and ready-to-use inactivated vaccine, adjuvanted by aluminium hydroxide and purified saponin. Its use is foreseen in emergency situations to combat infections caused by serotypes 2 and 4 strains of BTV. A dose of one-ml is recommended to be administered by subcutaneous route in sheep. 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. According to current European legislation, studies have to 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 Guideline on minimum data requirements for an authorisation under Exceptional circumstances for vaccines for emergency use against bluetongue (EMEA/CVMP/IWP/220193/2008) either representative experimental batches or standard production batches can be used. The guideline, 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, can be used to fulfil safety requirements. In the same document is mentioned that field trials are not strictly required. With reference to BTVPUR AlSap 2-4, the applicant carried out laboratory trials in sheep using batches of experimental vaccines formulated to contain higher antigen amount. Supportive data were also generated with vaccine preparations formulated with different payload of a Bluetongue serotype 9 (BTV9) antigen (in presence or not of BTV2 and BTV4 antigens) and with similar amount of adjuvants. Safety of an overdose and of repeated administration of one dose/overdose was further investigated using representative batches of the current vaccine. Data were provided to support the safety of the vaccine in cattle, although the product is not currently indicated for this species. All batches of vaccines used in the various safety trials (with the exception of those produced during early stage of development) were manufactured in accordance with the quality requirements presented in the analytical part of the present dossier.

A. Safety assessment

Laboratory tests

Safety experiments were carried out in the target animal species, e.g. in sheep but also in cattle which are not currently included in the indications of the vaccine. Conventional, BTV-antibody free-animals were used for all studies with the exception of one trial in which derived colostrums deprived cattle were used. Some proof of evidence of the safe use of the vaccine was provided from use of the vaccine in the field in Corsica and Portugal.

Preliminary work included supportive antigen dose studies using experimental and production batches of monovalent (BTV2 or BTV4) and of experimental bivalent BTV2&4 vaccine preparations. Additional support to safety was gathered from a study, as part of an experimental work carried out on BTV2&4 vaccine at Laboratorio Central De Veterinaria in Portugal or were extrapolated from a series of trials performed with different payload of BTV9 vaccine antigen combined with BTV2/4 vaccine antigens. Pivotal studies were also carried out using experimental and production batches of the current vaccine. In general, studies were performed under GLP conditions or according to Standard Operating Procedures (SOP) developed by the applicant and in compliance with current legislation. Local and general tolerance to vaccination was studied after each administration of the vaccine. Standard parameters used for supporting the safety profile of the vaccine were as follows:

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 sero-neutralising antibodies.

In general, the observation parameters and methodology adopted by the applicant were suitable for demonstrating the safety profile of BTVPUR AlSap 2-4.

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

Sheep

Safety of the administration of one dose

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 adjuvant payloads.

Systemic and local reactions observed in this study were, in general, acceptable. However, the data were 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).

Sheep were randomly distributed in 7 groups identified by a digit (1-7) of which 6 (constituted animals to be vaccinated and 1 (n.7) the control sheep.

The characteristics of the three inactivated experimental BTV4 vaccines, formulated with different amounts of antigen; of the experimental inactivated bivalent BTV2&4 vaccine are shown below:

Treatment group	**1**	**2**	**3**
Antigen	BTV4	BTV4	BTV4
Antigen/dose	2ml/dose	4ml/dose	8ml/dose
Treatment group	**4**	**5**	**6**
Antigen	BTV2&4	BTV2	BTV4 (MLV*)
Antigen/dose	4ml+4ml/dose	4ml/dose	Min. 4 (log_{10} $CCID_{50}$/dose

* Modified live virus

Results:

Clinical observations: No general reaction to vaccination was recorded.

Rectal temperature (T°): very few of the vaccinated sheep presented transient and moderate hyperthermia after vaccination. All sheep in group 6 presented a transient (lasting 1-2 days) hyperthermia. Significant difference ($p<0.05$) was registered between this group and all the remaining groups, while no statistical significant difference was registered between control group and other vaccinated groups.

Local reactions: frequency of local reactions to vaccination was low. Local reactions were never recorded in control group and in group 5 (BTV2 production batch). In one occasion (D6) a 3 cm^2 lesion was recorded in one sheep of group 4 (exp. BTV2&4 vaccine). The maximum number of observation of reactions at injection site was recorded on D0+4h. On D7 lesions were recorded in 20% of sheep in groups 2 (exp. monovalent BTV4 vaccine) and in 40% of sheep of group 6 (MLV vaccine). Min/maximum surface size (in cm^2) ranged from 1 to 9. Average sizes in the two groups were 0.2 and 2 cm^2 respectively. Average size of maximum local reactions ranged from a minimum of 0 cm^2in group 5 to 3.4 cm^2 in group 6.

Conclusions:

The general safety of the vaccine was demonstrated in that clinical signs were not strictly attributable to vaccination and hyperthermia induced by vaccination considered acceptable and not differing from that recorded in control animals. Local reactions, in terms of global frequency of occurrence and size were considered acceptable. However the study was considered of supportive nature as the batches used were experimental ones.

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. Overall data generated from the experimental vaccine preparations used in this study were taken into account to support the safety of the current vaccine.

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.
Sheep were randomly distributed in 5 groups (A to E) of animals to be vaccinated and 1 group (F) of control sheep that remained untreated.
The characteristics of the four experimental vaccines (B,C,D,E) formulated with different payload of pilot batches of BTV4 antigen that were produced are presented below:

Treatment Group	A	B	C	D	E	F
Antigen dose eq	4ml	2ml	0.4 ml	2ml	0.4 ml	___

Results: in few sheep, including controls the rectal temperature was ≥40.5°C. In all groups the maximum increase of rectal T° was recorded on D0+4h. Local reactions were recorded in 40% vaccinated animals. The minimum/maximum surface size (in cm^2) ranged from 0.25 to 12 (very small number of animals) . The reactions were of limited duration (maximum 6 days).

Conclusions: Local and general reactions recorded in this study were in general, compatible with an acceptable level of safety of the vaccine preparations. Information and overall conclusions from this study were used to support the modifications introduced into the manufacturing process of BTV vaccines.

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

i) Safety assessment of two BTV bivalent inactivated vaccines BTV1 & 4 and BTV 1 & 8 containing high antigens payload in one-month old lambs.

The objective was to assess the safety after administration of a double dose followed by two single doses of two bivalent inactivated vaccines formulated at high antigen payloads in one-month lambs.

Administration route and vaccine scheme:
One-month-old lambs were randomly allocated in 3 groups. Groups 1 and 2 constituted the vaccinated groups, vaccinated with BTV1&4 or BTV1&8 respectively. Group 3 constituted the placebo (control) group. On D0, each lamb received 2 ml (2x) of vaccine or placebo as appropriate for the group, by

subcutaneous route; on D14, each lamb received 1 ml of vaccine or placebo by the same route and on D29, each lamb also received the third vaccination of 1 ml of vaccine or placebo again subcoutaneously.

Results:
A moderate and transient rectal temperature increase was observed. No general reactions, except very rare and transient apathy were seen. Moderate swelling reactions appeared after the first and second vaccination that almost disappeared on D49, and more severe local reactions after the third vaccination due at particular site of injection (inguinal region). A classical local subcutaneous lesion was observed whereas there was no impact on body weight.
Conclusions:
Overall, the safe use in sheep of minimum age of the vaccine under application can be supported by the results obtained from the above study.

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

The objective was to evaluate the safety of an overdose administration of a monovalent BTV4 vaccine in sheep.

Administration route and vaccine scheme:
Animals were randomised in 2 groups (vaccinated and controls).

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

Results:
Clinical observations: no systemic reaction was reported in controls whereas sporadic (on D3 and D4) cough was recorded in one vaccinated sheep.

Rectal temperature (T°): in the two groups, maximal average increase of rectal T° was recorded on D0+4h. Differences were not statistically significant.

Local reactions: reactions at injection site were only recorded in all vaccinated sheep. The largest reactions (from 1-12 cm2, 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 which indicated the safety of the vaccine even in case of errors in dose administration.

iii) Safety assessment of a BTV2&4 vaccine formulated with a high antigen payload, following administration of repeated doses to 3-month-old-sheep
The objective was to evaluate the safety of an overdose and of a repeated (over)dose administration of an experimental batch of BTV2 &4 vaccine in sheep.
Sheep were randomly allocated in two groups (vaccinates and controls).
A BTV2 & 4 vaccine was used which contained per 1 ml dose: 2x of BTV2 and BTV4 and 2.7 mg/ml aluminium hydroxide.

Observation scheme and post-vaccination follow-up

The rectal temperature of each sheep was recorded. The animals were examined for local reactions, clinical signs, body weight. A macroscopical histopathological inspection of injection sites also took place.

Clinical observations: no systemic reaction was reported in controls whereas sporadic (on D3 and D4) cough was recorded in one vaccinated sheep.

Rectal temperature (T°): No statistical difference between controls and vaccinates was recorded after the first vaccination but statistically significant difference were recorded after the second vaccination and a similar trend ws observed after the third.

Local reactions: Reactions at the injection sites were recorded in all vaccinated sheep. Over the entire observation period, the size of reactions ranged from 0.3 to 4 cm^2. Maximum average sizes were 1.4, 1.3, and 0.7 cm^2 after each of the 3 vaccinations, respectively. No lesion was observed on D49 at post mortem inspection of each of the 3 injection sites in control animals, as well as of the injection sites in all vaccinated sheep after 1st and 2nd vaccination and of the injection sites in 90% of vaccinates after the third vaccination. In 10% of vaccinated sheep a granuloma of approximately 0.125 cm^3) was recorded. A granulomatous inflammation type lesion (maximum size: 0.6 cm^2) was only diagnosed by histopathological examination of the injection site of 30% vaccinated sheep.

Conclusions:
The repeated administration of the vaccine preparation did not induce general abnormal clinic reactions, and had no impact on growth performance. Repeated vaccination of sheep resulted in a transient and moderate hyperthermia, and acceptable lesions at injection site.

Cattle

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

The objective was to evaluate the safety of an inactivated experimental BTV2 monovalent vaccine in cattle (supportive information). 7-13-months old calves were used.

Local and general reactions observed in this study were, in general, acceptable.

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

The objective was to evaluate the safety of (repeated) administration of one dose of a production batch of bivalent BTV2&4 vaccine in cattle. On D0, and subsequently, on D28, two to two and a half months old calves received the assigned treatment (1 ml of vaccine or placebo as appropriate for the group) by subcutaneous route on (D0) and (D28).

Results:
Maximum increases of rectal temperatures ranged from 0-1°C and 0.3-1.2°C after the 1st and 2nd vaccination. The increases were transient (less than 24 hours). No local reactions were reported after the 1st vaccination. In 57% of calves a local reaction was recorded starting 24 on the first day after second vaccination. In 75% of these animals local lesions disappeared by D28.

Conclusions:
Local and general reactions observed in this study were, in general, acceptable.

iii) 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

The objective was to evaluate the safety in calves of an overdose and of a repeated doses administration of an experimental batch of BTV2&4 vaccine manufactured to contain a double content per dose of each of BTV2 & 4 vaccine antigen.

Administration route and vaccine scheme:

Calves were randomly allocated in 2 groups identified as vaccinated and controls. 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): dose injected: 2ml (double dose) of the vaccine
2nd vaccination (D14): dose injected: 1ml (1 dose) of the test preparation
3rd vaccination (D28): dose injected: 1ml (1 dose) of the test preparation

Control calves were treated with placebo solution on the same days, with the same dosage, route and site as vaccinated animals.

The rectal temperature (T°) of each calf was recorded. The animals were examined for local reactions, clinical signs, body weight.

Results:
No systemic reactions were reported in vaccinated or control calves. No nstatistically significant difference in the average maximum rectal temperature increase between controls and vaccinates after both vaccinations was observed. 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 reactions ranged from 1 to 28 cm^2. After 2-3 weeks, 1 cm^2 local reaction was still present in 20% of vaccinates. Following the second vaccination 60% of vaccinated calves presented local reactions. The 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 vaccinated calves.

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 following an overdose or repeated administration of one dose of the vaccine as they were limited to some swelling at the injection site (frequently associated with a transient swelling of the draining lymph node) that rapidly reduced within 1-3 week.

Examination of reproductive performance

i) Safety of a bivalent BTV2& 4 vaccine with high antigens payload in pregnant ewes.

This GLP compliant study was designed in order to assess the safety in pregnant ewes of a bivalent experimental batch of BTV2 & 4 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), each of primiparous or multiparous ewes, at approximately 7 or 18 weeks of pregnancy (at vaccination), were randomly allocated to the 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 and BTV4 antigens, 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 (apathy, loss of appetite, polypnea, salivation and tremor), 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. The presence of serum neutralizing antibodies to BTV2 and BTV4 was investigated in serum samples collected from blood samples taken from all ewes on D0 and D77 (before vaccination) and then at weaning.

Results:

A small number of abortions occurred in the control group, in ewes of group C, and D and of which half were 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 temperature was recorded in vaccinated animals. No statistically significant difference was observed between groups for rectal temperatures.

The total and mean numbers of born alive and dead lambs observed in each treatment group were similar. Some lambs died immediately after birth which was considered an expected result.

No statistically significant difference was observed between groups. Only non specific lesions were observed at necropsy of aborted lambs. For each ewe the total weight of weaned lambs (Kg) was calculated. 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 of the lambs observed in each treatment group was similar. There was no statistically significant difference between groups. Serological results were presented and discussed. With the exception of two ewes from control group, all remaining animals were negative to sero-neutralising antibodies against BTV2 and BTV4 antibody. While ewes from placebo group remained seronegative at lambing, a moderate and partial seroconversion was observed in the vaccinated 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.

ii) Safety of a bivalent BTV4 & 8 vaccine with high antigens payload in pregnant cows.

This study was designed in order to assess the safety in pregnant cows of a bivalent experimental batch of BTV4 and BTV8 vaccine formulated with high antigens payloads. The vaccine was administered to pregnant cows at different stage of pregnancy.

Study design:

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.Cows and heifers , 3-9 years at different stage of pregnancy and antibody negative to BTV4 and BTV8, were enrolled in the study. The animals were assigned to two treatments groups (G0, included controls, and G1, included vaccinated cows). 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 BTV4 and 2x BTV8 antigens per 1-mL dose. Each cow in G0 received physiological saline the same way as in G1.

Follow up:
The safety of the vaccine was assessed through a monitoring of the clinical signs (general reactions including rectal temperature) during four days following each vaccination, the reproductive performance (calving data and health status of the calves until 15 days of age) and the milk production.

Results:
The results of the study allowed demonstrating the safety in pregnant and lactating cows of two administrations of one dose of a bivalent BTV4 & 8 inactivated vaccine formulated with high antigens payload with:

- very limited and transient temperature increase following the first vaccination,
- absence of treatment-related general reactions,
- absence of effect on the milk yield in the cows that were in lactation at the time of vaccination and during the four following months,
- absence of impairment of the reproductive performance of the vaccinated cows whatever the stage of pregnancy at which the vaccine was administered

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 and during lactation. These results have also been considered by CVMP supportive of the use of the product in ewes.

Examination of immunological functions

No specific study has been carried out however there is no reason for suspecting an 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 were not provided. In several occasions the applicant referred to the safe use of BTV4 and/or BTV2&4 in Corsica, Spain, Portugal and Italy. In light of the current requirements in the CVMP Guideline on minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against bluetongue (EMEA/CVMP/ /IWP/ 220193/2008), field studies may be omitted.

B. Residue assessment

The vaccine contains inactivated whole virus, a buffer solution and adjuvant. The latter consists of aluminium hydroxide, saponin and water for injection. No specific residues studies were considered necessary.

MRL

The active substance being a principle of biological origin intended to produce active immunity is not in the scope of Regulation (EC) 470/2009.

The following ingredients of BTVPUR AlSap 2-4 suspension for injection for sheep are included in Table 1 (Allowed substances) of the annex to Commission Regulation (EU) No 37/2010 as follows:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Aluminium hydroxide	Not applicable	All food producing species	No MRL required	Not applicable	No entry	No entry
Saponin	Not applicable	All food producing species	No MRL required	Not applicable	No entry	No entry
Glycine	Not applicable	All food producing species	No MRL required	Not applicable	No entry	No entry

In addition to the above constituents the product contains the following excipients: phosphate buffer, silicon antifoam and water for injections. These ingredients, as used in this product, are considered as not falling within the scope of Regulation (EC) No 470/2009.

Withdrawal period

Species: Sheep: Zero days

Environmental Risk Assessment

A phase I assessment was carried out, providing evidence that there would be no potential risk for the global environment. No phase II assessment was deemed necessary. No hazard should be posed to the environment in light of the nature of the vaccine, in particular of the antigen (inactivated) and adjuvant(s) (appearing to be pharmacologically inert substances). Additionally, no special concern is posed by the final product in light of the safety of packaging, of the limited number of injections and of the maximum quantity administered to animals, of the route and of the method of administration, and disposal. Consequence and level of risk are practically nil, this justifying the absence of phase II assessment.

User safety

For the user there is a risk of self injection. Appropriate warnings and advice on the SPC would serve to reduce this risk.

Overall conclusions on safety

Potential risks arising from the use of BTVPUR AlSap 2 & 4 in sheep were tested in laboratory studies aiming to demonstrate the safety of the subcutaneous injection of experimental preparations and production batches of the vaccine administered in either single, repeated or (over)dose. All safety studies carried out in sheep were either GLP or compliant with the applicant's SOP. One study was provided in which animals used were of minimum age (1 month lambs). The safety studies were described in sufficient detail, thus providing clear evidence of the safety profile of the vaccine

preparations used in each trial. Under the tested conditions, vaccine preparations were generally well tolerated as demonstrated by the absence of major systemic reactions impacting body temperature and growth performances following administration in sheep or cattle. In general, local reactions in sheep were acceptable in terms of size, frequency of occurrence, and duration. Higher injection site reactions, remaining nevertheless acceptable, were recorded in cattle. However, at this stage the vaccine will not be indicated for these species. The safety of the vaccine in pregnant ewes was considered acceptable. Evidence was provided that showed that there is no potential risk for the environment. For the user there is a risk of self injection. Appropriate warnings and advice on the SPC would serve to reduce this risk.

4. Efficacy assessment

BTVPUR AlSap 2-4 is indicated to prevent infection, viremia and clinical signs in sheep caused by BTV serotypes 2 and 4. A dose of one-ml of the vaccine is recommended to be administered by subcutaneous route in sheep. 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. Onset of immunity was set at 4 and 5 weeks after primary vaccination course for BTV4 and BTV2 respectively. The duration of immunity has not yet been established but relevant studies will be provided.

Although efficacy studies were not performed in breeding animals, its use is not contraindicated in this category of target animal species. Based on the recommendations provided in the CVMP Guideline on minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against bluetongue (EMEA/CVMP/ /IWP/ 220193/2008), either representative experimental batches or standard production batches can be used. Both type of vaccine preparations were used in efficacy trials. With the exception of those produced during early stage of development, all batches of vaccines used in the various efficacy trials were manufactured in accordance with the quality requirements presented in the analytical part of the dossier. Batches of experimental vaccines formulated to contain lower antigen amount were tested in laboratory trials carried out in sheep. In all studies the efficacy of vaccines was tested in conventional, BTV-antibody free-animals.

The assessment of efficacy was established based on clinical and virological data as well as on immunogenicity. The level of protection was evaluated through the analysis and follow up of the effects of the inoculation of an infective dose of homologous BTV strains isolated from recent outbreaks in EU. In general, for this purpose, hyperthermia and titre/duration of viraemia produced after virus inoculation/challenge were considered the most objective parameters to be monitored in order to assess the course of infection. The level of viraemia was analyzed by either a classical virus isolation method, and/or BTV specific quantitative real time RT-PCR assay. Data were provided in order to support the efficacy of the vaccine also in cattle, although the vaccine is not currently indicated for this species. Field trials were not strictly required for this type of application and the applicant didn't provided any data related to them. DIVA strategy has not been implemented yet.

Laboratory studies:

Establishment of a Challenge Model

The characteristics of the virus inoculum used in laboratory studies and its relevance to the epidemiological situation, are of particular importance and were discussed by the applicant. The biological properties of field and laboratory adapted strains of BTV are markedly different. In sheep, reproduction of clinical signs with some serotypes and isolates may be difficult, the mortality rate and severity of clinical signs varying with the breed, age the type and strain of the virus and certain interaction with the environment of the animals infected. Bovines although susceptible to BTV develop

mainly a subclinical infection and a long lasting viraemia, this representing the only parameter normally tested under experimental conditions for this species.

The effect of factors like virus dose, type of inoculum, route of inoculation, passages in cell cultures, were evaluated during preliminary studies carried out by the applicant in order to establish a valid challenge model for BTV2 and BTV4.

Diagnostics techniques in the above studies included Sero-neutralisation (SNT), ELISA, RT-PCR and virus isolation. Overall, experimental conditions established by the applicant in order to demonstrate the efficacy of BTVPUR AlSap 2-4 vaccine were consistent with current knowledge and there was sufficient evidence to support that the challenge model used was acceptable.

Dose titration study

Sheep

i) Assessment of efficacy, by vaccination and challenge in sheep, of vaccines formulated with two different BTV-2 antigen batches

The objective was to assess, through a BTV2 virulent challenge, the efficacy afforded by six BTV2 vaccine preparations formulated with 2 different antigen batches and tested each at three different payloads. The study also intended to establish a relation between protection and antigen payload.

Study design:
Sheep of approximately 4.5 months of age sero-negative to BTV at vaccination were randomly distributed in 7 groups (A to G) and vaccinated by subcutaneous route with 1ml dose on D0 (groups A to F). Group G remained without treatment.

Animals in all groups were challenged with the virulent BTV2 challenge suspension on D35 after vaccination.

Materials: Vaccine (s)/Placebo

Antigen payload/1 ml vaccine dose			Group
Infective Titre	Volume (ml)	VP7 ELISA Titre	
6.81	1.83	10.0	A
5.81	0.18	1.0	B
4.81	0.02	0.1	C
7.21	1.93	10.0	D
6.21	0.19	1.0	E
5.21	0.02	0.1	F

Post-vaccination follow-up:
Clinical observation: sheep were monitored for rectal temperature, general condition, body condition and other clinical signs from D40 to D49.

Serology: blood samples were collected on D0, and frequently thereafter to determine specific BTV-2 antibodies by sero-neutralisation test.

Viraemia: blood samples were collected on D40, D42, D44, D47 and D49 to determine viraemia by qRT-PCR.

Results:
Efficacy results:
Serology: All sheep were BTV-2 sero-negative before vaccination, and the control sheep remained sero-negative until challenge. In the groups vaccinated with low antigen payloads, all but one sheep

were sero-negative on D21 as controls. In the group vaccinated with medium antigen payloads, slight sero-conversions were observed in most animals on D21. There was a tendency for each of these groups to be different from the control group. In the groups vaccinated with higher antigen payloads, all sheep had clearly seroconverted on D21. Each of these groups was significantly different from the control group. A statistical comparison was conducted with the 4 vaccine preparations containing medium and higher antigen payloads that demonstrated a significant antigen payload effect and an absence of batch effect.

Clinical signs: In general, all control animal registered clinical signs (principally congestion and oedema on the head). Several sheep of groups B, C and F presented clinical signs whereas in groups A, D and E, almost no sheep displayed significant clinical signs. Following a comparison it was indicated a difference between the batches at the medium antigen payload. These results are consistent with an effect related to payload as an infectious higher titre in CCID50 before inactivation (group E) provided a better protection than the lower titre (group B).

Viraemia: No data was presented concerning the viraemic status of the animals on the day of challenge. After challenge, all control animals were highly positive from D40 to D49. Virus peaked on D44 with an average value of 8.55 $\log_{10}$ RNA copies/ml blood and remained constantly high until D49. In groups C and F all sheep were found viraemic at least once during the study and most of the sheep remained viraemic until D49. Animal in groups B had similar virus titres to those observed in groups C and F. Only one sheep at one time point (D49) was not viraemic. Conversely, viraemia was prevented in 80% sheep in group E. In groups A and D virus was never detected in any animal. The complete prevention of viraemia observed in these groups was significant.

Conclusions:
Vaccines formulated with an antigen payload at least equal to group A payload, induced a significant increase of BTV sero-neutralising antibodies, provided significant clinical protection after challenge, and completely prevented viraemia in 100% of vaccinated sheep. The study demonstrated that vaccine's protection was strongly correlated to the antigen payload (infectious titre of virus culture before inactivation). The above were acceptable.

ii) Efficacy in conventional sheep, of BTV-2/4 vaccines formulated with different antigen payloads, against a BTV-4 virulent challenge

The objective was to assess, through a BTV4 virulent challenge, the efficacy afforded by two bivalent BTV2 &4 inactivated vaccine preparations.

Study design:
Sheep of approximately 4-5 months of age at vaccination (seronegative to BTV) were randomly distributed in 2 vaccinated groups (G1 and G2) and one control group (G3). They were subcutaneously vaccinated with 1ml dose on D0 (groups 1 and 2). Group G3 remained without treatment.

Materials: Vaccine (s)/Placebo

Vaccine batch	Payload of antigen per 1ml dose of vaccine	
	BTV-2	BTV-4
Group 1	0.05	0.16
Group 2	0.10	0.31

Animals in all groups were challenged with the virulent challenge strain on D21 after vaccination.

Post-vaccination follow-up:
Sheep were monitored frequently for rectal temperature general condition, body condition and other clinical signs (related or not to BTV infection)after challenge.

Serology: blood samples were collected on D0, D21 (before challenge) and D35 to determine specific BTV2 and BTV4 antibodies by sero-neutralisation test.

Viraemia: blood samples were collected on D21 (before challenge), and frequently thereafter to determine viraemia by qRT-PCR.

Results:
All sheep were BTV2 and BTV4 sero-negative before vaccination, and the control sheep remained sero-negative until challenge. All the sheep were confirmed to be BTV4 negative before challenge.

Hyperthermia: An increase of rectal temperature was observed in the control group, starting at D28 and peaking on D29 then progressively reducing up to D35. Maximum hyperthermias were significantly lower in both vaccinated groups G1 and G2 when compared to control group G3.

Clinical signs: After challenge, the most frequently observed clinical signs were congestion and oedema of lips, nostrils and intermandibular space. Skin erythema, nasal discharge or crusts and cough were also occasionally observed. The frequency and the duration of observation of these signs were significantly higher in controls group G3 compared to both vaccinated groups.

Viraemia: In G3 60% of sheep were positive on D26 (i.e. 5 days after challenge), thereafter, from D28 until the end of the monitoring period, viral genome was detected in all control sheep, at high titres. In G1 20% of the sheep was positive from D26 to D33 whereas in G2 all the animals remained negative. Reduction in G1 or prevention in G2 of viraemia was statistically significant. No sero-conversion against BTV2 was observed in G1 and G2 at any time points after vaccination or BTV4 challenge. Few sheep in G1 and G2 slightly sero-converted after vaccination, whereas all sheep, whatever the group, sero-converted after challenge.

Conclusions:
The efficacy of the two vaccine preparations under test was demonstrated against BTV4 challenge through a significant reduction of hyperthermia, a significant reduction of clinical signs and a significant reduction (G1) or prevention (G2) of viraemia. The applicant provided practical justifications for not using animals of minimum age (e.g. difficulties to find lambs of the appropriate age, and, in addition to this, due to the epidemic of BTV8 over the past years, difficulties in finding 1 month-old sero-negative lambs). The applicant considered that the immune response to vaccination of susceptible 1-month-old young ruminants should not be considered different from the one of susceptible adult ruminants (4-5 months of age) also in view of the results of efficacy studies with a similar vaccine of serotype 8 (BTVPUR AlSap 8). The above were considered acceptable.

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

The objective was to evaluate the immunogenicity and the protection provided by a production batch of the current vaccine in sheep.

Study design:
Sheep were randomly distributed in 4 groups (identified by letters A to D corresponding to the treatment group). Animals in groups A and B were used as controls, and sheep in groups C and D were vaccinated with the test vaccine. Sheep in groups A and C and sheep in groups B and D were challenged respectively by BTV2 and BTV4 virulent strains.

On D0, each sheep of groups C and D was subcutaneously injected with 1ml/dose of the test vaccine.

Post-vaccination follow-up:
Blood samples for detecting virus neutralizing antibody to BTV2 and BTV4 antibody were taken on D0 (vaccination), D14, D21, D28 (challenge) and on D42.

Challenge:
Twenty-eight days after vaccination (D28), sheep in respective groups (vaccinated and controls) were separated (A/C and B/D) and challenged with a virulent suspension:
A/C: strain BTV2
B/D: strain BTV4

Post-challenge follow-up:
Clinical observation: sheep were monitored for rectal temperature, general behaviour and conditions and for specific signs of BTV daily from D33 (e.g. 5 days after challenge) to D42.

Viremia: blood samples were collected starting five days after challenge (D33), and frequently thereafter.

Results:
Clinical monitoring:
Maximum hyperthermia: Controls in A group had higher maximum hyperthermia than vaccinates in group C and similarly controls in group B had higher values than vaccinates in group D.

Global Clinical Scores (including T°): Controls in group A had much higher scores than vaccinates in group C and similarly values in controls in group B were higher than vaccinates in group D.

Virological findings: In control group A (BTV2 challenge) all sheep were found positive throughout the observation period after challenge while vaccinated sheep of group C were never found positive. In control group B (BTV4 challenge) 67% of sheep were found positive throughout the observation period after challenge while vaccinated sheep of group D were never found positive.

Sero-neutralising antibodies (SNT): Sero-neutralising antibodies against BTV2 and BTV4 remained nearly unchanged in control animals until challenge, thereafter seroconversion was recorded. Neither BTV4 challenged controls (group B) showed BTV2 seroconversion nor BTV2 challenged controls (group A) showed seroconversion to BTV4 after respective challenge. Seroconversion was demonstrated in all vaccinated sheep although at nearly similar titers depending on the vaccine antigen and groups. After challenge, a strong increase of SNTs to both BTV2 and BTV4 was recorded in controls while only a slight increase of SN antibodies were recorded in vaccinated animals.

Conclusions:
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. As a consequence of the experimental conditions of this study, the onset of immunity in sheep could be set at 4 weeks following the completion of the primary course of vaccination in this target animal species.

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

The objective was 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.

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 control animals (C). Another group included animals vaccinated with alive attenuated vaccine.

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:
Animals were inoculated with the virulent challenge of a BTV 2 virus. Challenge was carried out 4 weeks after the first vaccination injection.

Post-challenge follow-up:
Clinical observation: rectal temperature, general behaviour and clinical signs were recorded prior to challenge and at frequently thereafter*Viremia:* blood samples were collected on the day of challenge (D42), and frequently thereafter.

Results:
All control animals presented severe changes of their general behaviour and hyperthermia. 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

In general, sheep of all vaccinated groups remained healthy without major changes in their body conditions or abnormal increases of rectal temperature. Sporadic signs of BTV were recorded in few vaccinated sheep. With the exception of one sheep 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 sero-neutralising antibodies after one injection, whereas the second injection had a booster. After challenge animals of control group seroconverted showing the highest individual and average sero-neutralising antibodies aginst BTV in 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 were considered within the context of a preliminary supportive work.

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

The objective was to evaluate the efficacy of the subcutaneous injection of 1ml/dose of six experimental vaccine prototypes containing different BTV2 antigen and adjuvants payloads.

Study design:
Sheep were randomly distributed in 7 groups (6 vaccinated groups V, W, X, Y, Z, E and 1 control group C). On D0, each sheep in its respective group was subcutaneously injected with 1ml/dose of the assigned vaccine preparation.

Post-vaccination follow-up:
Blood samples for detecting virus neutralizing antibody to BTV2 and BTV antibody by competitive (c) – ELISA (were collected on D0 (vaccination), D7, D14, D23 (challenge) (and then, on D37).

Challenge:
Twenty-three days after vaccination (D23), all sheep were challenged with the virulent suspension of BTV2.

Post-challenge follow-up:
Clinical observation: sheep were monitored for rectal temperature, general behaviour and conditions and for specific signs of BTV daily from D28 (e.g. 5 days after challenge) to D37. *Viremia:* blood samples were collected five days after challenge (D28), then on D30, D32, D35 and D37.

Results:
Clinical signs: challenge in controls was considered of moderate severity as far as clinical outcome is concerned. Therefore a comparative evaluation with results obtained in vaccinated animals appeared more appropriate. On average, maximum hyperthermia was higher than recorded in vaccinated animals. *Virological findings:* Viraemia was detected as soon as 5 days (D28) after challenge in 5 control sheep by both RT-PCR and virus isolation on embryonated chicken eggs. With the exception of animals in group "X", a small number of sheep of each of the remaining groups was tested positive at RT-PCR performed on D30. RT-PCR was negative in all other instances among vaccinated sheep.

Sero-neutralising ELISA antibodies: Sero-neutralisng antibodies against BTV2 remained unchanged in control animals until challenge, thereafter seroconversion was recorded. At D14, seroconversion was demonstrated in all vaccinated sheep at varying titres. On D23, at challenge, while all control animals were negative, 50% of vaccinated sheep, were found positive to BTV Elisa antibody detection. Fourteen days after challenge all sheep were tested positive.

Conclusions:
All vaccinated sheep were protected against hyperthermia, clinical signs and viraemia. In this study lack of correlation between sero-neutralising antibodies and protection was demonstrated. BTV ELISA antibody can not be used for assessment of vaccination/protection. However the results of this study can still be considered within the context of a preliminary supportive work.

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

The objective was 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. Six of those groups were vaccinates and one was controls. Three inactivated experimental BTV4 vaccines, formulated with different amounts of antigen were used; one inactivated bivalent BTV2&4 vaccine; one monovalent BTV2 inactivated; and a BTV4 attenuated modified live 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:
Blood samples for detecting virus neutralizing antibodies to BTV2 and BTV4 were collected at different time points after vaccination.

Challenge:
On the day of challenge (D29), sheep were challenged with 1 ml of the BTV4 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 sero-neutralising antibodies against BTV2 and BTV4 and BTV ELISA antibody were collected at different time points before and after challenge. *Viraemia*: blood samples were collected for detection of viraemia by RT-PCR 5, 7, 9, 12 and 14 days after challenge.

Results:

Clinical signs following challenge were 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 in 80% of vaccinates with a double dose of BTV2. Viraemia was never detected in any other vaccinated sheep. Sero-neutralising antibodiesagainst BTV2 remained unchanged in control animals until challenge, thereafter seroconversion was recorded. BTV2 vaccinated sheep had no BTV4 sero-neutralising antibodies, whereas in BTV4 vaccinated sheep, homologous seroconversion was observed as early as at D7. Sheep vaccinated with a live modified BTV4 vaccine had higher scores of homologous antibodies. With the exception of sheep of the attenuated vaccine, a clear anamnestic response to challenge was observed in all vaccinated animals to levels of antibodies similar or even higher than those recorded in controls animals after challenge.

Conclusions:

The administration of vaccines formulated with higher amounts 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 BTV2 vaccinated sheep, neither clinical nor virological protection was obtained. The CVMP considered that the conclusions from this study can be taken into account in the context of preliminary supportive work as batches used were experimental ones.

vii) Assessment of efficacy of four monovalent BTV4 vaccines formulated with antigen batches produced according to two processes

The objective was to evaluate the efficacy of BTV4 experimental vaccine preparations formulated with different payloads of pilot antigen batches that were produced with two processes.

Study design:

Sheep were randomly distributed in 5 groups (A to E) of animals to be vaccinated and 1 group (F) of control sheep as control for the challenge experiment. On D0, each sheep in a specific group (with the exception of control sheep) was subcutaneously injected with 1ml/dose of respective vaccine preparation.

Treatment Group	A	B	C	D	E	F
Antigen dose eq	4ml	2ml	0.4 ml	2ml	0.4 ml	___

Challenge:

On the day of challenge (D23), sheep were injected with the virulent suspension of BTV4.

Post-vaccination follow-up:

Clinical observation: Clinical signs and rectal temperature were monitored frequently after challenge.

Viremia: blood samples were collected for detection of viraemia by qRT-PCR 5 (D28), 7 (D30), 9 (D32), 12 (D35) and 14 (D37) days after challenge.

Results:

Clinical monitoring: Clinical signs that were most frequently observed, whatever the group, were primarily in the form of congestive and swelling lesions of several regions of head and skin erythema. Maximum peaks of hyperthermia in most controls were recorded between 5-10 days after challenge.

Virological findings: Viraemia was detected by qRT-PCR as soon as 5 days after challenge in 67% control sheep. These animals remained positive throughout the monitoring period. From the remaining sheep 2/3 were positive. Besides one sheep, viraemia was never detected in any other vaccinate.

Serology: Sero-neutralising antibodies against BTV4 remained unchanged in control animals until challenge, thereafter seroconversion was recorded. In BTV4 vaccinated sheep, homologous seroconversion was already observed by D14 but tended to decrease at challenge. A slight increase of SNTs in response to challenge was observed in all vaccinated animals to levels similar to those recorded in controls animals after challenge.

Conclusions:

The 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.

Cattle

i) Assessment of efficacy of a bivalent BTV2&4 inactivated vaccine by vaccination and challenge in cattle

The objective was to evaluate the efficacy of a production batch of bivalent BTV2&4 vaccine by vaccination/challenge against BTV2&4 in cattle.

Study design:

Four to five 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 /Controls BTV4 /Vaccinates BTV2/Vaccinates BTV4.

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 with a viral suspension of BTV2 or BTV4.

Post-challenge follow-up:

Rectal T° and appearance of clinical signs were recorded daily for 14 days after challenge. Blood samples were collected for serology testing on D65 before challenge, and thereafter on D86. Serum samples were tested by competitive ELISA (c-ELISA). Blood samples for detection of viraemia were collected on D65, prior to challenge, and thereafter, three times/week for the next 42 days.

Results:

Vaccination resulted, although at slightly different extent, in seroconversion to BTV2 and BTV4 respectively in almost all vaccinates. Seroconversion to both BTV serotypes was only recorded in control animals after challenge (D86). After challenge, none of the vaccinated animals developed detectable levels of viraemia. Conversely, BTV2 was detected in the blood of all control animals starting from 3 days after challenge and lasting 16 days. For both serotypes the highest peak of viraemia was observed on D75, e.g. 10 days post challenge.

Conclusions:

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. However a

clear anamnestic response associated to viraemia was only recorded in controls. Conversely, only a slight increase of sero-neutralising antibodies was recorded in vaccinated cattle, this result in line with the finding of absence of viraemia in these animals. As a consequence of the experimental conditions of this study, onset of immunity in cattle should be set at 5 weeks following the completion of the primary course of vaccination in this target species.

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

The objective was 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.

Study design:
Seven to 13-months old calves were enrolled. On D0, clinical examination was carried out and rectal T° was recorded from all calves which were then injected subcutaneously, with a 1 ml/dose of the vaccine. 1ml/dose of the vaccine was injected again 21 days later, subcutaneously.

Post-vaccination follow-up:
On D0, D14, D21, D28 and D35 blood samples for serology were collected.

Results:
Al animals were sero- negative to BTV2 and to BTV ELISA antibodies before vaccination. In some calves seroconversion was already observed 21 days after the 1st vaccination. Fourteen days after 2nd vaccination all calves had homogeneous levels of sero-neutralising antibodies at high values. At D21 (3 weeks after 1st vaccination) all calves were negative to BTV antibody detection by ELISA. From seven days after the 2nd vaccination (D28) in all calves ELISA antibody were detected (72% of inhibition).

Conclusions:
Uptake of the vaccine was demonstrated, thus allowing some supportive conclusions to be made for the efficacy of the current vaccine in cattle

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

The objective was to evaluate the immunogenicity of a production batch of bivalent BTV2&4 vaccine by measuring sero-neutralising antibodies against BTV2 and BTV4 in cattle.

Study design:
On D0 each cattle was injected subcutaneously with a 1 ml/dose of the vaccine. One ml of the vaccine was injected again 28 days later (D28) subcutaneously.

Post-vaccination follow-up:
On D0, D14, D21, D28, D35 and D42 blood samples for serology were collected.

Results:
All animals were considered sero -negative to both BTV2 and BTV4 before vaccination. Eighty percent sero-converted to BTV2 (at titers considered as low-moderate) 21-28 days after the 1st vaccination. Conversely, at D21 time point, individual sero-neutralising antibodies against BTV4 were never below 0.48 and on D28 in only 20% of vaccinated animal, a level that was considered as low. Fourteen days after the 2nd vaccination all calves had detectable levels of sero-neutralising antibodies against BTV 2 and BTV 4.

Conclusions:
Uptake of the vaccine was demonstrated, thus allowing some supportive conclusions to be made for the current vaccine from this study.

iv) Assessment of immunogenicity of a BTV2&4 inactivated vaccine in young cattle

The objective was to evaluate the serological response following primary course vaccination of calves with a production batch of bivalent BTV2&4.

Study design:
Calves of 2-2.5 months old were randomly allocated in 3 groups . Groups 1 and 2 were vaccinated. The remaining animals (group 3) constituted the placebo (control) group. 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 (D0) and (D28).

Post-vaccination follow-up:
Blood sampling for evaluating the immunogenicity of the vaccine by measuring SNTs to BTV2 and BTV4 was performed on D-1, D14, D21, D27, D35 and D42.

Results:
At the time of second vaccination, almost all calves had seroconverted to both vaccine antigens. A booster effect towards both vaccine antigens was already recorded seven days after second vaccination (D35), the calves showing similar SNTs to BTV2 and BTV4. On D42 SNTs had increased for both BTV2 and BTV4.

Conclusions:
The immunogenicity of the test vaccine was demonstrated.

Influence of maternal antibody on the efficacy of the vaccine

No specific study was performed to investigate the impact of pre-existing maternally derived antibodies to the vaccine's efficacy. 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.

Duration of Immunity

Sheep

i) Assessment by serology and challenge, of the duration of immunity (DoI) afforded by an inactivated BTV2 vaccine, administered in 1, 2, or 3 injections

The objective was to evaluate the protection afforded against a homologous virulent challenge carried out either 6 or 12 months after 1, 2 or 3 course vaccination regimen using a monovalent BTV2 vaccine.

Study design:
Sheep were randomly distributed in groups designated as controls (g.1/C) and vaccinates (g.2/"V1" and 3/"V1"+"V2"), and thereafter sub-distributed in order to have the following groups at challenge

Groups	C	V1		V2	
Subgroups		V1	V1+ V3	V1+ V2	V1+ V2+V3
D0 (V1)	No	Yes		Yes	
D21 (V2)	No	No		Yes	
D182(V3)	No	No	Yes	No	Yes
Challenge at D182	Yes	Yes	____	Yes	____
Challenge at D364	Yes	Yes	Yes	Yes	Yes

On D0, D21 and D182 each sheep in its respective group was subcutaneously injected with 1ml/dose of vaccine preparation.

Post-vaccination follow-up:
The health status during the vaccination phase was regularly assessed. Blood samples for detecting virus neutralizing antibody to BTV2 were collected at different time points after vaccination/at challenge/after challenge.

Challenge:
On the day of challenge (D182 or D364), sheep in respective groups were challenged with a BTV2 virulent suspension.

Post-challenge follow-up:
Clinical observation: after the respective challenge, sheep were monitored for rectal temperature, general behaviour and conditions and for specific signs of BTV (global clinical score) daily, starting from 5 up to 14 days after challenge. Necropsy of all sheep was performed the next day.

Viremia: blood samples were collected for detection of viremia by qRT-PCR, 5,7,9 12 and 14 days after each challenge.

Results:
Challenge after 6 months (g.V1/V1+V2 vs Controls):

Maximum peaks of hyperthermia were recorded between 7-9 days after challenge. On average, higher values were recorded in controls and lower values in vaccinates for groups V1 and V1+V2. Clinical signs were mostly observed in controls and were primarily in the form of congestive and swelling lesions of several regions of head, and of the so called "butterfly sign"(only observed in controls, lasting several days and occasionally accompanied with skin erythema in the inguinal region). On average, the highest clinical score in control animals was recorded 8 days after challenge. Most controls presented oral ulcers compared to sheep in group V1. None of the sheep of group V1+V2 presented this kind of lesions.

Challenge after 12 months (g.V1/V1+V2/V1+V3/V1+V2+V3 vs Controls):

Maximum peaks of hyperthermia were recorded between 6-9 days after challenge. On average, maximum hyperthermia in controls was higher than in vaccinated animals. Clinical signs mostly observed in controls were primarily in the form of congestive and swelling lesions of several regions of head, and of the so called "butterfly sign. On average, the highest clinical scores were recorded in controls than in vaccinated animals. Common findings of lesions at necropsy were nearly similarly recorded in all groups. Petechias on the spleen were only found in vaccinated sheep. Five out of seven controls presented an average of 4.0 (ranging from 1-10) oral ulcers. This lesion was only sporadically recorded in vaccinated sheep.

Validation of challenge by virological findings: Viraemia was detected by qRT-PCR as soon as 5 days after D182 and D364 challenge in all control sheep. Viraemia was never detected in vaccinated sheep.

Sero-neutralising antibodies */ELISA antibodies:* sero-neutralising antibodies against BTV2 remained unchanged in control animals until challenge, thereafter seroconversion was recorded. At D20, seroconversion was demonstrated in all vaccinated sheep at similar levels.

Conclusions:
Information gathered from this study can only be considered within the context of a preliminary supportive work. The applicant has committed to perform additional DoI studies in sheep with BTVPURAISap 2 &4.

Field trials

Data on field trials were not provided. In light of the current requirements in the CVMP Guideline on minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against bluetongue. (EMEA/CVMP/ /IWP/220193/2008), field trials may be omitted.

Overall conclusion on efficacy

The efficacy of the vaccine was demonstrated in laboratory conditions reproducing moderate to severe challenge in target animal species. Relevance to the current epidemiological EU situation of the challenge was demonstrated by selecting relevant strains among field isolates from recent outbreaks of BTV in EU. In addition, as no significant antigenic variation is yet documented among BTV2 and BTV4 strains currently circulating in Europe, the strains present in BTVPUR AlSap 2-4 vaccine can still be considered suitable for the production of effective vaccines against these two serotypes of BTV.

The main parameter used to substantiate the efficacy of the vaccine was the absence of detectable viraemia post-challenge, associated where relevant, with an overall prevention of clinical signs. In this respect, the detection system played a major role, the combination of virus isolation/titration on eggs and RT-PCR shown to provide the most suitable tool for the virological follow up of animals after challenge.

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 duration of immunity has not been established and the applicant is required to provide the results of relevant studies.

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

5. Benefit risk assessment

Introduction

BTVPUR AlSap 2-4 is an inactivated vaccine conventionally produced, liquid and ready-to-use, adjuvanted by aluminium hydroxide and purified saponin. It is indicated to prevent infection, viremia and clinical signs in sheep caused by BTV serotypes 2 and 4. The product is a bluetongue vaccine and as such in view of the epidemiological situation and the lack of authorised products is being considered as an application for authorisation under exceptional circumstances.

Benefit assessment

Direct benefits

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

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

Clinical trials demonstrated that the product is capable indeed of inducing an immune response which prevents viraemia and reduces clinical signs in sheep. The effect would be to prevent transmission and minimise the impact of clinical signs.

Additional benefits

BTVPUR AlSap 2-4 is a standard inactivated vaccine and as such fits in with accepted vaccination practices in the field.

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

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

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

Risk assessment

Main potential risks:

- A small local swelling at the injection site (at most 24 cm^2) can be observed following vaccination for a short period (at most 14 days). A transient increase in body temperature, normally not exceeding an average of 1.1°C, may also occur within 24 hours after vaccination.
- For the user there is a risk of self injection. Appropriate warnings and advice on the SPC would serve to reduce this risk.
- For the environment there is negligible risk that the vaccine components may cause unexpected effects to the environment.
- For the consumer there are no components which require an MRL, therefore there are no concerns regarding MRL. The product contains components found in other marketed products and therefore the risk is no greater than already exists.

Specific potential risks, according to product type and application:

- Limited data are available on the duration of immunity. As a result an appropriate revaccination programme cannot be recommended at this stage.
- Limited data are available on the stability of product during storage. It is permissible for a preliminary shelf life of 12 months to be granted for this product due to its exceptional nature. Nevertheless there is a risk that the product may not be stable for this period.

Risk management or mitigation measures

- Appropriate warnings have been placed in the SPC to warn of the potential risks to the target animal, end user and environment.
- In addition the risk to the environments is considered minimal because the antigen is inactivated and adjuvant(s) appear to be pharmacologically inert substances. Additionally, no special concern is posed by the final product in light of the safety of packaging, of the limited number of injections and of the maximum quantity administered to animals, of the route and of the method of administration, and disposal. The consequence and level of risk are minimal, justifying the absence of phase 2 assessment.

Evaluation of the benefit risk balance

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

The formulation and manufacture of BTVPUR AlSap 2-4 are largely well described and specifications are supported. The applicant is able to detect sub-potent batches thereby ensuring that the product of consistent quality will be produced.

It is well tolerated by the target animals and presents a low risk for users and the environment and appropriate warnings has been included in the SPC. A zero days withdrawal period has been set.

Conclusion on benefit risk balance

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

- Bluetongue disease is spread by insect vectors and therefore presents particular challenges in terms of control due to an inability to prevent transmission from infected animals other than through insect control combined with reducing or preventing viraemia (virus in the blood) in susceptible animals by means of vaccination.
- Bluetongue disease is epizootic in nature and has the potential to result in high morbidity and mortality in susceptible populations, particularly of sheep.
- The remaining epidemiological risk from Bluetongue serotype 2 (BTV2) and serotype 4 (BTV4) for European sheep populations, in view of recent and previous outbreaks of BTV2 and BTV4 in Europe constitutes an objective need to have authorised products available for use in the coming months.
- Consequently any delay should be avoided where possible in making available safe and effective vaccines that have been demonstrated to be in compliance with the CVMP Guideline on minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against bluetongue (EMEA/CVMP/IWP/220193/2008).

Conclusion

Based on the original and complementary data presented 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, data on the stability and duration of immunity of the vaccine should be provided as stated in the specific obligations of the opinion and 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. Based on the original and complementary data presented, the CVMP concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 2001/82/EEC.