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Veterinary Medicines and Product Data Management

Scientific discussion

This module reflects the initial scientific discussion for the approval of Bovilis BTV8 (as published in September 2010). For information on changes after this date please refer to module 8.

1. Summary of the dossier

Bovilis BTV8 is an inactivated vaccine presented as a suspension for injection, intended for the active immunisation of sheep and cattle for the prevention of viraemia in sheep from 1 month of age and the reduction of viraemia in cattle from 6 weeks of age, caused by Bluetongue Virus, serotype 8. The active substance of Bovilis BTV8 is the inactivated Bluetongue Virus (BTV) serotype 8. The vaccine dose is 1 ml. Primary vaccination in sheep includes injection of a single dose whereas in cattle two injections administered with an interval of approximately 3 weeks are needed. The benefit of Bovilis BTV8 is the stimulation of active immunity in sheep and cattle against bluetongue virus serotype 8, resulting in prevention of viraemia in sheep and reduction of viraemia in cattle. The extent of this reduction has been shown by epidemiological modelling studies to be likely to reduce virus transmission to an extent that can limit the spread of an outbreak in a vaccinated population. The most common side effects are a slight rise in temperature (usually not more than 0.5°C, in individual cases up to about 2°C) for up to three days after vaccination, and temporary swellings at the injection site. In sheep these swellings typically last for up to three weeks. In cattle small palpable swellings may still be present up to six weeks after vaccination in approximately one third of the vaccinated animals.

BTV can cause intense disease outbreaks in sheep. Fever is the most usual but not invariable clinical sign. If fever occurs sheep first become pyrexic 4-10 days after infection. Acute form in sheep is usually characterised by pyrexia up to 42°C, depression, emaciation, ulceration of the oral cavity, swollen and sometimes cyanotic tongue, excessive licking movements of the tongue, lameness and abortion. Infection may result in the death of sheep within approximately 8-10 days or in a long recovery period with negative impact on the animals' welfare and growth. Mortality rate in sheep could reach up to 70% in a flock. Although BT is less common in cattle, some clinical signs have appeared in recent epizootics in Northern West Europe caused by the BTV 8 serotype. The most prominent lesions in BTV-8 infected cattle included nasal discharge, crusts/lesions of the nasal mucosa, salivation, fever, conjunctivitis, dysphagia, depression, congestions of the oral mucosa, redness of the skin, swollen teats and lameness.

Over the last ten years, the bluetongue situation in the EU has considerably changed with incursions of new serotypes, particularly in the last two years of serotype 8 into an area of the EU where outbreaks have never been reported before and which was not considered at risk of bluetongue. Recent outbreaks due to serotype 8 occurred in the Netherlands, in Belgium, Germany, Luxemburg, France

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and in the UK. It is considered likely that the disease will remain in Europe for the next few years creating an endemic situation.

The dossier was reviewed in line with the provisions of Article 39(7) of Regulation (EC) No 726/2004 for an authorisation under exceptional circumstances and the recommendations of the CVMP Guideline on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/IWP/220193/2008).

2. Quality assessment

Composition

Composition for dose of 1 ml is provided in the following table.

Names of substance		Quantity per 1 ml dose
Active substance	Inactivated BTV serotype 8 antigen	500 antigenic units/ml prior to inactivation*
Adjuvant	Aluminum hydroxide	16.7 mg
Adjuvant	Purified saponin	0.31 mg
Excipients	Trometamol	
	Sodium chloride	
	Maleic acid	
	Simeticone emulsion	
	Water for injection	

(* inducing a virus neutralising antibody response in chickens of $\geq 5.0 \log_2$)

Container

The vaccine is filled in 10, 20, 50, 100, 200, 250 and 500 ml polyethylenterephtalate (PET) containers. The containers are closed with a nitryl rubber stopper (Eur. Ph 3.2.9) and sealed with a coded aluminium cap.

Development Pharmaceutics

The vaccine strain selected for the BTV8 component was isolated in 2006 from an infected cow located in the southern part of the Netherlands. Further details about the isolation, passaging and testing were provided. Baby Hamster Kidney cells were infected with the BTV working seed and cultured. At the end of the culturing step the propagated virus was collected and inactivated by binary ethyleneimine (BEI), according to a system which is similar to that already in place for other proprietary inactivated vaccines. Selection of the optimal combination of the antigen and adjuvant system was primarily based on the results obtained from a preliminary dose/response-vaccination/challenge experiment carried out in lambs. Animals were vaccinated with prototype vaccines. Subsequently the lambs were challenged with pathogenic BTV-8. It was established that the vaccine should contain at least 500 antigenic units (AU) per ml. Moreover, based on the results obtained and the experiences with other vaccines in ruminants, the aluminium-saponin combination adjuvant was also selected for the BTV vaccine. Although variations may occur in the sequence of different BTV field isolates, at present stage the choice of the vaccine strains was sustainable. The production and control systems should not pose any major concern both relying on processes and methodologies well established by the applicant.

The applicant explained that in the production campaign of 2008 several batches of the vaccine that were formulated to consistent antigen content were produced at two manufacturing sites. All batches are subject to the same set of final product tests and the results obtained from these tests confirm that the manufacturing process is robust and generates consistent vaccine batches independent of the production site. In this respect, the manufacturer's batch protocols and summary of relevant testing were also presented of three consecutive batches of finished product per site. The results were also presented of the potency data of several batches. Overall, evidence of the consistency of production was provided. The applicant provided information on batches of antigen produced at both production sites in order to address how equivalence of manufacture for the 2 manufacturing sites is taking place.

Composition of the batches used in the clinical trials

Data were provided for batches used in the safety and efficacy trials carried out under laboratory conditions, in line with the provisions 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). Any deviations of the batches used in the safety and efficacy trials from the proposed standard vaccine batch were addressed satisfactory. The batches used in the GLP safety as well as the efficacy trials represented a worst case.

Method of manufacture

The manufacturing process takes place in two phases: A) production of the vaccine antigen and B) production of the finished product. Detailed flow chart(s) were presented and outlined the steps taken in the production of vaccine antigen and of final product and the control tests performed on the corresponding stage of production.

The stages of the manufacturing process were described and all the operations were conducted in conditions of sterility and according to GMP and GLP principles.

Manufacture of vaccine antigen

Virus is grown in BHK-21 cells. Expansion of the initial cell seed is carried out in order to obtain the amount of cells needed to cultivate the vaccine virus. Clean cell cultures are inoculated with BTV8 working seed at an appropriate multiplicity of infection. The culture is incubated to obtain a sufficient amount of the virus. Thereafter cells are disrupted to release the antigen and tested by virus titration to ensure that the maximum allowed viral titre will not be exceeded. In addition samples are taken for determination of the antigen content. The suspension undergoes an inactivation process. Samples are taken from the inactivated and neutralized bulk and are tested for sterility, the presence of residual thiosulphate and for inactivation. The results of testing carried out on three antigen batches produced at both production sites were provided.

Manufacture of the finished product

The bulk vaccine is prepared by blending pre-determined amounts of one or a mixture of several batches of inactivated and neutralized BTV-8 antigen, adjuvants and excipients. Details of the sequence followed and the maximum time required for blending each component and final mixture were provided.

The blending strategy is based on antigen content per dose of 500 AU (as determined from the antigenic mass ELISA on virus harvest). Antigen batches are selected based on the antigenic mass and can be pooled if necessary.

The bulk vaccine is mixed well and stored at 2-8°C until filling. The final product is stored at 2-8°C and shelf life is expected to be 12 months.

The results of testing carried out on three consecutive batches of vaccine were provided and were acceptable.

Validation studies

Antigenic mass ELISA

The ELISA was validated according to the relevant quality VICH guidelines. Specificity, linearity, robustness, repeatability, and intermediate precision of this test were sufficiently demonstrated.

Inactivation kinetics of BTV8 antigen

In order to test the inactivation kinetics of BTV8 antigen, two study reports were presented. In both cases inactivation kinetics studies were carried out on pilot batches of non-concentrated or concentrated live bulk antigen. The obtained results met the requirements of the Ph. Eur and allowed defining of the maximum pre-inactivation titre that is applicable to the inactivation method. In addition, the results obtained at large scale production confirmed those obtained at pilot scale.

Validation of inactivation control test

The validation of the inactivation control test on BTV8 bulk antigen produced at both sites was provided. The results of the sensitive inactivation control tests showed that no live virus was detected in the inactivated samples, independent of the size of antigen harvest.

Other validation data

The validation and the analytical description of the control test for the determination of residual sodium thiosulphate in BTV8 vaccine and of aluminium (ions) in the finished product were provided and were satisfactory. Validation data were also provided concerning the sterility test carried on finished product. Demonstration was provided that the test is carried out according to Ph. Eur. The reports concerning suitability of the potency test were presented as well.

Control of starting materials

Listed in a Pharmacopoeia

The Certificates of analysis (CoA) of the tests carried out by the applicant in order to guarantee the quality of all the starting materials in compliance with the requirements of Eur. Ph. were provided and were satisfactory. These starting materials are presented in the following table.

Starting material	
Trometamol	
Glycerol	
Hydrochloric acid, concentrated	
Sodium hydroxid	
Sodium chloride	
Water for injection	
Sodium thiosulphate	
Maleic acid	
Dimethyl sulfoxid	
Aluminium hydroxide (gel), hydrated,for absorption	

Not listed in a Pharmacopoeia

Starting materials of biological origin

The following starting materials of biological origin were used in the preparation of the vaccine, as follows:

Starting material
BHK-21 cells (Boxmeer)
BHK-21 cells (Cologne)
BTV8 clone 1
Bovine serum
(Porcine)Trypsin
Lactalbumine hydrolysate
Tryptose
Tryptose phosphate broth (TPB)

Certificates of analysis of the testing carried out by the applicant for the above materials were provided.

Cell substrate(s) used for the production of BTV8 clone 1 antigen

The applicant justified its intention to produce the BTV8 antigen at two manufacturing sites, Boxmeer (The Netherlands) and Cologne (Germany). Vaccine antigen is produced in BHK-21 cells. The absence of relevant extraneous agents in the Master Cell Stocks (MCS's) of BHK-21 cells was documented in accordance with the applicable EU legislation.

BTV-8 clone 1 Master Seed Virus (MSV) BTV

The vaccine strain originated from a cow during an outbreak in the Netherlands. Blood was collected from this animal and used to isolate BTV8. BTV8 infection was confirmed by the Central Institute for Animal Disease Control in Lelystad in September 2006, and later on by the reference laboratory of the Institute of Animal Health in Pirbright, UK. Later in the year 2006, the animal was transferred to Intervet. The obex was collected and sent to the Dutch reference laboratory for TSE at Lelystad, to test the animal for the presence of prions. The cow was diagnosed negative for BSE. The treatment of the infectious material and the production of the MSV were described in detailed. A working seed stock can be prepared at any level between MSV+1 and MSV+4 by propagation of the preceding virus level in BHK-21 cell culture. MSV has been tested for bacterial and fungal sterility and freedom from mycoplasma (by culture and indicator cell methods) according to Ph. Eur. Requirements. The absence of relevant extraneous agents in the MSV was fully documented in accordance with the applicable EU legislation. In order to minimize the potential risk associated with the absence of testing for some relevant contaminants, an overall risk assessment concerning the use of (all) starting materials of biological origin was provided.

Bovine serum

Blood serum from bovine foetuses, newborn calves or adult cattle is used as component of cell culture medium. Assurance that the donor animals comply with the regulations concerning TSEs was supported by the provision of EDQM certificates of suitability. Overall the reduction factors observed for the different viruses (including adenovirus and parvovirus), taken together, were considered sufficient to guarantee the viral purity of the final product and to justify the limited testing for adventitious viruses.

(Porcine)Trypsin for cell culture

Trypsin of porcine origin and lactose monohydrated (derived form bovine milk) is used in the manufacturing process. It is tested by the supplier for the absence of salmonella, mycoplasma and porcine parvovirus and details of the country of origin are provided on the certificate. Details of suppliers and TSE statements were included.

Lactalbumin hydrolysate for cell culture

This is an enzymatic digest of lactalbumin which is extracted from milk whey after removal of casein. The applicant confirmed that only suppliers meeting TSE requirements will be used. The supplier provided a certificate showing the country of origin, and the tests performed and their results. A CoA was included. Details of suppliers and TSE statements were provided.

Tryptose

This is a mixed enzymatic hydrolysate of porcine (enzyme) and bovine (milk) origin. Only suppliers meeting TSE requirements will be used. A CoA was included. Details of suppliers and TSE statements were provided.

Tryptose phosphate broth (TPB)

This is a buffered dehydrated medium used for cultivation of fastidious micro-organisms. Its composition is as follows: Tryptose, Dextrose, Sodium chloride & disodium phosphate. Only suppliers meeting TSE requirements will be used. A CoA was included. Details of suppliers and TSE statements were provided.

Saponin

Saponin is isolated from the dried bark of the South American tree *Quillaia saponaria* Molina and consists mainly of a mixture of vegetable glycosides optimised and purified for use as an adjuvant in veterinary vaccines. A CoA was provided.

Starting materials of non-biological origin

2-Bromo-ethylamine-hydrobromide (BEA)

BEA is a white to nearly white crystalline substance well soluble in water. Under slightly alkaline conditions in water dissolved BEA reacts to become active binary ethyleneimine (BEI). It is obtained from professional suppliers. A certificate of analysis was provided.

Simeticone emulsion

Simeticone emulsion is useful for defoaming aqueous systems (antifoam). It is an oil-in-water emulsion. A CoA as well as TSE-risk assessment were included.

In House preparation of media

Basal medium

A standard cell culture media for BHK-21 cell culture is used, and may be supplemented with tryptose (phosphate broth) and lactalbumin hydrolysate.

Culture medium for cells and virus production

Basal medium may be supplemented with bovine serum and a mixture of antibiotics.

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

The assessment of the starting materials was conducted according to the Note for Guidance (NfG) on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01), 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 (EMEA/CVMP/019/01). In this respect, a declaration of compliance with the NfG was provided by the applicant. An overall assessment of the risk of transmission of TSE by Bovilis BTV8 according to the NfG was provided. In addition, a declaration was provided (by the specialized laboratory of the Central Institute for Animal Disease Control in Lelystad The Netherlands) concerning the negative results obtained from two tests carried out to exclude the presence of prions in relevant materials derived from the cow from which BTV8 seed material was isolated. The assessment was conducted in order to demonstrate that the risk of transmission of TSE is significantly minimised by: i) documenting and recording the sourcing of animals, ii) the nature of animal tissues used in manufacturing (of low or no detectable infectivity), iv) the production processes, and by the negligible risk posed by a series of further factors which would likely lower the risk if any, such as high dilution of the materials used, route of administration and maximum number of dosage injected. Satisfactory certifications of suitability or conformity of the materials used were provided.

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.

Control tests during production

The aim of control tests during production is to check antigenic mass content, completeness of BTV8 inactivation, residual sodium thiosulphate in the inactivated antigen, sterility, filling. Details for each are provided below. Details of the tests including the methods and pass criteria were provided and were considered satisfactory. In summary the following main tests are performed during production:

a) the antigenic mass content

b) the complete inactivation of BTV8

c) the residual sodium thiosulphate in the inactivated antigen

d) the sterility of the (concentrated) antigen

e) filling

Results were provided for three consecutive batches of vaccine antigen produced at the two different sites at formulation stage and were acceptable.

Control tests on the finished product

The aim of control tests on the finished product is to check sterility, safety, and potency (and identity), physical aspects (e.g., visual appearance and pH), and assay of adjuvants (aluminium and saponin content).The tests carried out on the finished product are summarised below:

Test:
Sterility test
Target animal safety test (in calves)
Identification and assay of active ingredients: *In vivo* potency and identity test using chickens
General characteristics of the finished product: visual appearance
General characteristics of the finished product: Determination of pH
Determination of aluminium content
Determination of saponin content

Consistency of production

The applicant presented results from test on 3 consecutive batches. Equivalence and consistency of production has been satisfactorily demonstrated at the two proposed sites.

Batch potency test

The finished product is formulated on the basis of a fixed antigen content measured on the harvest before inactivation by means of a validated ELISA for antigenic mass determination. The applicant developed a batch potency test on the final product which is capable to discriminate between sub-potent and potent batches. The applicant explained that an in vivo test in Specific- Pathogen Free (SPF) chickens was chosen as animal model because it is well standardised, readily available and already used for other proprietary inactivated vaccines. The optimisation and validation of the potency test contributed to demonstrate the consistency of production (within the sites and between the sites) and the efficacy of each batch of vaccine that is released.

The steps followed by the applicant in order to ensure a suitable batch potency test, are the following:

1: optimisation of the test system in SPF chickens and subsequently

2: demonstration that the optimised test can discriminate between sub-optimal and standard batches

3: setting the batch release requirement

4: validation of this batch release

The applicant provided studies investigating the dose response in the proposed optimised potency test, and concluded from these that the proposed potency test would be capable to distinguish between a full dose and ¼ dose. Furthermore, vaccination-challenge trials in sheep and cattle with a batch containing 4 times reduced antigen content showed a similar level of protection as compared to the animals vaccinated with a standard batch of vaccine (500 AU/ml).

Stability

Stability of the bulk antigen

Data on the stability of the bulk antigen and of the finished product were not yet available. On the basis of the submitted data an interim shelf life for the antigen for 12 months was accepted pending provision of full stability data.

Stability of the finished product

The applicant indicated that real-time stability programme for both the finished product and antigen were initiated.

The applicant provided data from three batches produced in Boxmeer and 4 batches produced in Cologne, which have been tested after 0, 9 and 15 months storage. The product remained within specification for the period of observation. It was accepted as supporting the 12 months shelf life for an emergency vaccine. The timing for the provision of the results related to the proposed final product filled in PET containers as well as the bulk antigen was provided.

As a result of the above the CVMP concluded that a shelf life for the finished product of 12 months can be granted for an authorisation under emergency circumstances, pending provision of full stability data.

Conclusions on stability:

A shelf life for the finished product of 12 months can be granted and the provided stability results were noted. The applicant has committed to provide results from relevant stability studies.

OVERALL CONCLUSION ON QUALITY

The applicant has provided data in support of the method of production, in-process and finished product testing and stability of the product. The equivalence of antigen production at the two proposed sites and the new potency test and proposed specifications were considered acceptable also in view of the relevant post authorisation commitments listed in the corresponding section of the report.

As a result all the data and clarification provided by the applicant can be considered sufficient for granting a marketing authorisation under exceptional circumstances, when taking into account the benefit-risk balance for BTV serotype 8, and when considering the epidemiological situation in the EU.

In this context given that:

- a batch with minimum antigen content was shown efficacious on sheep and cattle,
- the production process allows production of consistent batches,

then the CVMP has sufficient guarantees to assume that forthcoming batches will be efficacious on sheep and cattle when manufactured and released on the basis of the descriptions and specifications laid down in this file.

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

3. Safety assessment

Bovilis BTV8 is a whole-virus, conventionally produced, liquid and ready-to-use, BEI inactivated vaccine, adjuvanted with aluminium hydroxide and saponin (from Quillaja Saponaria). Final batches of the vaccine are formulated in order to contain in a 1ml dose, 500AU (measured prior to inactivation) of the inactivated BTV8 strain, and an amount of respectively, 16.7 mg of aluminium hydroxide and 0.31 mg of purified saponin. Its use is foreseen to combat infections caused by serotype 8 strains of BTV. The vaccine is intended to be administered subcutaneously to sheep and cattle, from 1 month of age for sheep and from 6 weeks for cattle, in order to stimulate an active immunity capable to prevent viraemia in sheep and to reduce viraemia in cattle caused by infection with BTV8.

In sheep the vaccination schedule consists of a single administration of a 1ml dose, in cattle a second administration should be given three weeks after the first dose. According to the current European legislation, studies should be performed to demonstrate the safety of a vaccine for target animals of the youngest age for which the vaccine is intended to be used, and, if the vaccine is intended to be used in breeding animals, examination of the reproductive performances has also to be carried out.

Quantification of the antigen content is a pre-requisite for appliance of the minimum data requirements outlined 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). Additionally, it provides the basis for compliance with provisions of Annex I Part 7 of Directive 2001/82/EC, which requires that *the dose used* (in safety study) *is that quantity of the product to be recommended for use and containing the maximum titre or potency for which the application is submitted*. Based on these provisions, the safety studies presented below can be considered in compliance with the current EU legislation. The deviation noted for the minimum age of sheep (1.5 months on average instead of 1 month) is considered of less impact for the whole compliance of this part of the dossier. Results from safety studies in breeding animals, primarily in pregnant animals were provided and summary reports from field trials.

A. Safety assessment

Laboratory tests

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

Sheep

Safety study with inactivated whole virus vaccine against BTV serotype 8 in 5-10-week-old susceptible lambs after subcutaneous administration of an overdose followed two and four weeks later by a single dose

Objective/ Methodology

To evaluate the safety of the administration of a volume of 2ml (corresponding to an overdose) and of repeated (2x) administration of one dose (1ml) of the above vaccine, in sheep.

Summary of the experimental design

Approx 1.5 month old lambs were vaccinated by the subcutaneous route with a volume corresponding to an overdose (2ml) of the vaccine (D0). A single dose (1ml) was administered to each of these animals two (D14) and four weeks (D28) later. At the same times, additional lambs were injected with a placebo (Phosphate buffered saline solution (PBS)) and kept as controls. Lambs were observed daily up to two weeks after the third vaccination (D42) for the occurrence of local and/or systemic reactions. Thereafter, animals were necropsied, sites of injection were macroscopically examined and relevant samples were taken for histological examination.

Materials: Vaccine/Placebo

A production batch was used to vaccinate the lambs of group 1. PBS 0.01mM was used as placebo.

Administration route and vaccine scheme

On D0 (on average, at approximately 45 days of age), and subsequently, two (D14) and 4 (D28) weeks later, each lamb received the assigned treatment as summarized in the table below.

Group	Treatment	Vaccination dose per lamb on			Route	Number of lambs
		D0	D14	D28		
1	Vaccine	2ml	1ml	1ml	sc	11
2	Placebo	2ml	1ml	1ml	sc	9

Observation scheme and post-vaccination follow-up

The health status of the animals was individually monitored prior to vaccination and, in particular, the lambs were observed for clinical abnormalities on D-1 and D0 and just before the first vaccination to determine the pre-treatment status. Thereafter, animals were checked daily for any abnormalities occurring in general appearance, behaviour, locomotion, appetite and respiration. Lambs were particularly checked for the appearance of acute adverse reactions during four hours after each administration of the vaccine. Body temperature was measured. The occurrence of local reactions was monitored daily throughout the whole period of the trial. Injection site was also inspected. In case of a local reaction, the size was measured (e.g. length x width in cm). At necropsy, the injection sites were carefully inspected to evaluate the extent and nature of the local reactions. Tissue samples were taken for microscopic examination, if local reactions were present.

Blood samples were collected before (T0) and 2 weeks after 3rd (T42) vaccination and tested for the presence of BTV ELISA antibodies and Virus Neutralisation (VN) antibodies.

Results

No vaccine-related systemic reactions were observed. One day after the first and second vaccination, rectal temperature (T°) in the vaccinated group was significantly higher than in controls animals. The max individual increase of rectal T° throughout the study was recorded in one lamb 1 day after vaccination (41.4°C); in this case rectal T° remained ≥40.5°C (e.g. 41°C) for 12 more days. All lambs showed local reactions during the experimental period. At necropsy, moderately sized local reactions were found in all vaccinated animals. The local reactions after the first vaccination (with the overdose) were comparable in size with those after the second and third vaccinations (single dose). Microscopically, a granulomatous inflammation was found in all animals.

At vaccination all lambs were found to be BTV8 antibody free by ELISA and VN test. Forty-two days after initial vaccination, all animals were BTV antibody positive by ELISA and average VN-titre was $6.1 \pm 1.1 \log_2$.

Conclusions

Overall, the vaccine was well tolerated by the animals, which only reacted with a temporary increase in body T° and a granulomatous inflammation at injection site(s) moderate in size and likely to disappear eventually or to lead to minor scar tissue. Overall, local and general reactions recorded in this study would be considered of acceptable nature and extension. The study was considered sufficient to support the safety of the product in sheep.

Calves

Safety study with an adjuvanted inactivated whole virus vaccine against BTV serotype 8 in 4-5-week-old susceptible calves after repeated subcutaneous administration

Objective/ Methodology

To evaluate the safety of the administration of a volume of 4 ml (overdose), followed by repeated (2x) administration of a volume of 2ml of the above vaccine, in calves.

Summary of the experimental design

Calves negative for BTV-antibodies and of approximately 32 days old were vaccinated by the subcutaneous route with a volume of 4ml of the vaccine (D0). A volume of 2 ml was administered to each of these animals two (D14) and four weeks (D28) later. At the same times, additional calves were injected with a placebo (PBS) and kept as controls. Calves were observed daily up to two weeks after the third vaccination (D42) for the occurrence of local and/or systemic reactions. Thereafter, animals were necropsied, sites of injection were macroscopically examined and relevant samples were taken for histological examination.

Materials: Vaccine/Placebo

A production batch was used to vaccinate calves of group 1; PBS 0.01mM was used for the placebo group.

Administration route and vaccine scheme

On D0 and subsequently, two (D14) and 4 (D28) weeks later, each calf received the assigned treatment as summarized in the table below.

Group	Treatment	Vaccination dose per calf on			Route	Number of calves
		D0	D14	D28		
1	Vaccine	4ml	2ml	2ml	sc	6
2	Placebo	4ml	2ml	2ml	sc	5

Observation scheme and post-vaccination follow-up

The health status of the animals was individually monitored prior to vaccination and, in particular, the calves were observed for clinical abnormalities on D-1 and D0 and just before the first vaccination to determine the pre-treatment status. Thereafter, animals were checked daily for any abnormalities occurring in general appearance, behaviour, locomotion, appetite and respiration. Calves were particularly checked for the appearance of acute adverse reactions during four hours after each administration of the vaccine. Body T° was measured. The occurrence of local reactions was monitored daily throughout the whole period of the trial. Injection site was inspected for possible thickenings just before the first vaccination. In case of a local reaction, the size was measured.

At necropsy, the injection sites were carefully inspected to evaluate the extent and nature of the local reactions. Tissue samples were taken for microscopic examination, if local reactions were present.

Blood samples were collected before (T0) and 2 weeks after 3rd (T42) vaccination and tested for the presence of both BTV ELISA antibodies and VN antibodies. Both, the serological status of the calves at first vaccination and the humoral response against BTV were measured.

Results

All vaccinated animals showed sero-conversion against BTV-8 in the BTV-8 ELISA at the end of the experiment.

No systemic reactions were observed after repeated vaccination with an overdose and repeated single doses of the inactivated BTV-8 vaccine.

One day after both first (4 ml) and second vaccination (2 ml), rectal temperatures were significantly increased as compared with the control animals. The individual increase in body temperature (T_{max} 40.4 °C) after vaccination lasted maximally until three days after vaccination.

All calves showed local reactions during the experimental period. The local reactions after the first vaccination (with 4 ml vaccine) were comparable in size with those after the second and third vaccinations (2ml) and consisted of a soft swelling that decreased in size and changed into small hard swellings. At necropsy, moderately sized local reactions were found in all vaccinated animals. Microscopically, a granulomatous inflammation was found in all animals.

Conclusions

The vaccine was well tolerated by the animals, which only reacted with a temporary increase in body T° one day after vaccination and a granulomatous inflammation at injection site(s) moderate in size and present for up to 6 weeks.

Overall local and general reactions recorded in this study would be considered of acceptable nature and extension.

Examination of reproductive performance

Study reports were provided from two safety trials carried out in pregnant sheep and cattle. The main features of these two GLP safety study are summarised below.

Sheep

Overdose safety test (subcutaneous administration) with Bovilis BTV8, in pregnant ewes

Pregnant ewes (at different stages of gestation) sero-negative for BTV-8 and BTV-RNA free at the time of vaccination were enrolled. At first vaccination, group 1 was in the first, group 2 in the second and group 3 in the third trimester of gestation. A vaccine batch containing twice the standard content of antigen was used. The animals were vaccinated subcutaneously with a double dose of the vaccine, followed 3 weeks later by the administration of a single dose of the vaccine.

Details of the treatment schedule are reported in the following table.

Group	Dosing (2 and 1 ml/dose) at pregnancy week	
1	6	9
2	10	13
3	14	17

Before each vaccination, the animals were checked for pregnancy. The animals were observed daily until the end of pregnancy. The length of the gestation period and the clinical health of the lambs were recorded. The ELISA and VN antibody response to vaccination was also monitored.

Results

Three weeks after the second vaccination all ewes showed sero-conversion as determined by both methods. No clinical abnormalities, systemic reactions to vaccinations or mortality were observed during the whole course of the study in any of the groups. Pregnancy outcome was equal to that obtained in practice. It was concluded that subcutaneous vaccination of pregnant ewes with Bovilis

BTV8 with an overdose of the vaccine, followed by a single dose, was well tolerated and had no effect on the outcome of pregnancy.

Conclusions:

Overall, the safety of the vaccine was demonstrated in ewes at different stages of pregnancy.

Cattle

Overdose safety test (subcutaneous administration) with Bovilis BTV8 in pregnant heifers

Pregnant heifers sero-negative for BTV-8 and BTV-RNA free at the time of vaccination were enrolled. At first vaccination, group 1 was in the first, group 2 in the second and group 3 in the third () trimester of gestation, respectively. A vaccine batch containing 500AU/ml was used for the first vaccination; a vaccine batch containing twice the standard content of antigen was used for the second vaccination. The animals were vaccinated subcutaneously with four doses of the vaccine batch used, followed, 3 weeks later by the administration of 2 doses of the other vaccine batch used. Details of the treatment schedule are reported in the following table.

Group	Dosing (4ml and 2 ml/dose) at pregnancy week	
1	9-13	12-16
2	14-23	17-26
3	27-36	30-39

Before each vaccination, the animals were checked for pregnancy. The animals were monitored for systemic reactions and observed daily until the end of pregnancy. The length of the gestation period and the clinical health of the heifers were recorded. The ELISA and VN antibody response to vaccination was also monitored. All animals were ELISA antibody negative at first vaccination and showed sero-conversion two weeks after the second vaccination. No systemic reactions during the period from vaccination until delivery were observed. Incidentally, acute local swellings were observed in a small number of animals, disappearing the next day. Neither relevant clinical abnormalities nor mortality were observed during the whole course of the study in any of the groups. Each heifer delivered one calf at term. In group 2, two calves died during parturition. Post-mortem examination and the interval between vaccination and parturition make a causal connection between the death of the two normal sized calves at parturition and vaccination with Bovilis BTV8 improbable. It was concluded that subcutaneous vaccination of pregnant heifers with Bovilis BTV8 with a 4 ml injection volume, followed 3 weeks later by the injection of a 2 ml volume of the vaccine with double antigen content, was well tolerated and had no effect on the outcome of pregnancy.

Conclusions:

Overall, the safety of the vaccine was demonstrated in cows at different stages of pregnancy.

Examination of immunological functions

There is no reason to suspect an impairment of the immune system due to the vaccination.

Interactions

The standard interactions statement is included in section 4.8 of the SPC. A recommendation for not mixing the vaccine with any other medicinal product is included in section 6.2 of the SPC.

Field Studies

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. However, additional data on the safe use of the current vaccine generated from any planned field trials would be considered of further support to the current application.

To supplement the numerous laboratory studies presented up till now, six additional reports describing the outcome of field studies performed by the applicant became available during the evaluation of the application. The overall results obtained from the six studies, showed that after vaccination with Bovilis BTV8, local reactions of limited size were observed in sheep, cattle as well as in goats but there was no influence on general health and feed intake. Milk yield was not affected in cattle and goats whereas in sheep only a small decrease was noticed. Sero-conversion 3 to 4 weeks after (last) vaccination (as determined by the competitive ELISA) depended on the farm and varied from 13%-59% and 73-100% in sheep and cattle, respectively. In case of goats, 92% of animals seroconverted at 2 weeks after the second vaccination. Since no outbreaks of Bluetongue occurred in any of the farms involved in the trials, it was not possible to draw direct conclusions about the clinical efficacy of the product. Based on the outcome of the mentioned above studies, it is concluded that vaccination with Bovilis BTV8 under field conditions is safe in sheep, cattle and goats.

The overall safety of the vaccine administered under field conditions was demonstrated in sheep, goats and cattle.

B. Residue assessment

Study of residues

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 following constituents of the intended product Bovilis BTV8 are included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010:

Pharmacologically active substance	Marker residue	Animal Species	MRL	Target Tissues	Other Provisions	Therapeutic Classification
Aluminium hydroxide	Not applicable	All food producing species	No MRL required	Not applicable	No entry	No entry
Quillaia saponins	Not applicable	All food producing species	No MRL required	Not applicable	No entry	No entry
Sodium Chloride	Not applicable	All food producing species	No MRL required	Not applicable	No entry	No entry

Simeticone	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: trometamol, maleic acid and water for injection. For the purpose and at the doses at which they will be administered, these excipients are considered as not falling within the scope of Regulation (EC) No 470/2009.

Withdrawal period

Species: Sheep: :Zero days

Species: Cattle: Zero days

Environmental Risk Assessment

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

No hazard should be posed to the global 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 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 therefore minimal, 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

The safety profile of the vaccine in both laboratory and field trials was established. The vaccine appeared to be overall safe from the minimum recommended of age for use both in lambs and calves respectively. It was also found safe for use in pregnant animals of both species. The potential for any adverse effects following the administration of the vaccine under the recommended conditions of use is adequately reflected in the relevant section of the SPC. 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

Bovilis BTV8 is a whole-virus, conventionally produced, liquid and ready-to-use, BEI inactivated vaccine, adjuvanted with aluminium hydroxide and saponin. The final batches of the vaccine are formulated to contain in a 1ml dose, 500AU (measured prior to inactivation) of the inactivated BTV-8. The vaccine is intended to be administered subcutaneously to sheep from 1 month of age and to cattle from 6 weeks of age, in order to stimulate an active immunity capable to prevent viraemia in

vaccinated sheep and reduce viraemia in vaccinated cattle caused by infection with BTV8 respectively. Onset of immunity is 3 weeks after completion of the basic vaccination course which consists of one single injection in sheep and a vaccination regimen with an interval of three weeks for cattle. Duration of immunity (DoI) has been established at 6 months although revaccination of animals is recommended at least two weeks before each risk period. The influence of maternally derived antibodies (MDA) on vaccine efficacy has not been studied yet, such an omission being reflected in a statement included in SPC. Field trials were not strictly required for this type of application. Compliance with provisions of Annex I Part 8 of Directive 2001/82/EC, and of Ph.Eur. 2005 (General monograph, Vaccinia ad usum veterinarium- Evaluation of efficacy of veterinary vaccines) requires that *the dose used* (in efficacy study) *is that quantity of the product to be recommended for use and containing the minimum titre or potency (expected at the end of the period of validity*) *for which the application is submitted*. The submitted trials were in compliance with the above requirements.

Laboratory studies

Results of three laboratory studies carried out with the aim to define critical parameters were provided in order to establish a reproducible challenge model for BTV 8 in sheep and consequently to demonstrate the efficacy of Bovilis BTV8 in sheep and cattle. Overall the PCR used by the applicant in the different studies presented was considered as satisfactory validated.

Establishment of a Challenge Model

Two experimental studies were carried out in order to evaluate factors like virus challenge dose, type of inoculum and passages in cell cultures in the establishment of a valid challenge model for BTV8 in sheep.

Development of challenge model in sheep to reproduce bluetongue by the administration of bluetongue virus serotype 8 (BTV-8)

Objective

The general purpose was to establish a challenge model for BTV8 in sheep in order to study the effect of different doses and inoculum on the onset, duration and description of clinical signs and on the onset and duration of viraemia. Clinical signs as well as viraemia induced by three different amounts of virulent BTV serotype 8 subcutaneously administered to sheep were examined.

Animals

Sheep, free of antibody to BTV were enrolled. Animals were randomly allocated to 5 groups (G1 to G5). Animals in G1 served as non-challenged controls and were kept in the farm of origin.

Challenge

The virus was isolated from a BTV8-infected sheep. BTV-8 was grown in chicken embryo and subsequently adapted to growth on susceptible cells. Sterile saline solution was used for the placebo treatment of sheep in G1.

Animals were inoculated with 1 ml of saline (G1) or with different amounts of the challenge material.

Follow-up

Efficacy parameters which were evaluated to consider the infection as successful included clinical signs relevant to BTV8, body temperature, serology, vireamia and mortality. Additional testing was carried out to detect ELISA BTV-specific and BTV-8 neutralising antibodies.

Results

Clinical monitoring: Only animals infected with the highest dose of virus developed fever, mild clinical signs of dyspnoea and conjunctivitis associated with viraemia.

Body temperature: Comparison of mean values of body temperature between viraemic and non viraemic groups showed statistically significant differences.

Viraemia: The viraemia was measured either by RT-PCR or Virus Isolation and the coincidence of results with both techniques was high. All animals challenged with the highest infectious dose were consistently found infected by day 6 post infection until the end of the trial. Only one out of four sheep challenged with the intermediate dose became infected.

Serology: Antibodies to BTV were developed only by those sheep that became viraemic, but with some differences.

Conclusions:

The effort to establish a challenge model capable to reproduce BTV8 infection in sheep was acknowledged, as well as the quality of the study and the large amount of information provided. However, the relevance of this information to the purpose of the current application was considered limited, namely for the experimental nature of the study. The extrapolation to cattle of any results obtained in sheep was considered questionable.

Evaluation of the efficacy of Bovilis BTV8 upon challenge with virulent BTV8 virus in sheep

Objective

In order to support the evidence that the development of clinical signs of BTV8 is dependent on the age of the animals involved in the experimental study, the applicant referred to another experiment, where the efficacy of Bovilis BTV8 to protect adult sheep against clinical signs and viraemia caused by a BTV8 experimental challenge was investigated.

Challenge

Different challenge parameters were varied and evaluated. Two different types of challenge virus strains were compared.

Animals

Adult instead of young animals of two different breeds were used. Animals were randomly allotted to five groups (G1 to G5).

Experimental design:

The animals of G1 and G2 were vaccinated subcutaneously once (D0) with one dose of Bovilis BTV8. The control groups, G3, G4, and G5 were treated on D0 with 1 ml of saline solution. Three weeks after vaccination, sheep of G1 and G3 and G2, G4 and G5 were challenged subcutaneously with the two different challenge materials, respectively.

Follow-up

Efficacy parameters which were evaluated included serology, vireamia, clinical signs relevant to BTV8, body temperature.

The overall conclusions of this trial showed that single vaccination with Bovilis BTV8 significantly reduced viraemia and clinical signs caused by BTV 8 infection in adult sheep. Both challenge virus strains were able to induce early and long lasting viraemia, plus typical clinical symptoms of BTV disease.

Dose titration study(ies) - Determination of the adjuvant and onset of protection in sheep

Sheep

Efficacy obtained after subcutaneous vaccination of sheep with different experimental BTV 8 serotype vaccines

Objective

The efficacy (and the general safety) of the subcutaneous injection of seven prototype vaccines formulated with different BTV8 antigen payloads/adjuvant systems, and administered in single and/or double vaccination regimen one or two doseswas investigated in sheep. To this aim clinical reaction index, vireamia and the humoral response were evaluated after challenge.

Animals

Sheep of 3 months of age (on average) were selected for the study. The animals were distributed in 8 groups.

Materials

Seven inactivated and adjuvanted prototype vaccines were used to vaccinate sheep subcutaneously. The table below provides information on these experimental vaccine preparations which were used at each group at 1 ml/dose.

Treatment schedule

Several vaccination schemes and formulations were evaluated. Animals in Group 1, 2, 3 and 7 were vaccinated on Day 1 and Day 22. Animals in groups 4, 5, 6 were vaccinated on Days. Controls of group 8 were injected with saline on Day 1 and on Day 22. All animals were challenged on Day 43.

Challenge

The challenge material consisted of virulent heterologous BTV-8 grown on susceptible cells.Challenge was carried out on D43, e.g. 3 weeks after one injection (D22) or two vaccination (e.g. D1 and D22).

Post-challenge follow-up

Efficacy parameters which were evaluated included serology, vireamia, clinical signs relevant to BTV8, body temperature.

Local adverse effects after vaccination were also monitored.

Results

No clinical signs attributable specifically to BTV were observed in any vaccinated animal. No increase of rectal temperature could be detected in any vaccinated sheep with two exceptions.

Viral RNA was not detected in any of the vaccinated animals no matter the type of prototype formulation used.

BTV specific and BTV8 neutralising antibodies were detected three weeks after first vaccination for almost all vaccine preparations. The administration of the challenge virus boosted the humoral response of all vaccinated animals.

Conclusions:

The CVMP concluded that this study offers evidence that a single vaccination in 10-12 week old sheep provides some protection against a field strain challenge at 3 weeks post vaccination. No viraemia was

detected in vaccinated, challenged sheep. However this study is considered only supportive to the efficacy data package of the vaccine.

Efficacy of subcutaneous vaccination of sheep with different doses of inactivated BTV serotype 8 formulated in saponin and Al(OH)3 in sheep

Objective

The aim of the study was to evaluate the efficacy of an experimental adjuvanted inactivated whole virus vaccine against BTV-8 in sheep. Posology (single vs. repeated vaccination) and antigen load per dose were the parameters tested.

Animals

Five-week-old lambs were divided in 8 groups. Animals of groups 1, 2 and 3 were vaccinated twice (Day 0 and Day 21) with three weeks apart with 1000, 500 or 250AU/ml, respectively. Animals of group 4, 5 and 6 were single vaccinated at day 21 with the same antigen doses. Two groups remained unvaccinated as controls.

Treatment schedule

Several graded doses and vaccination schedules were evaluated.

Challenge

Three weeks after the last vaccination, all sheep were challenged subcutaneously with virulent heterologous BTV-8 grown on susceptible cells. The virus was isolated from a BTV8-infected sheep from The Netherlands. All animals were challenged on Day 42.

Follow-up:

Efficacy parameters which were evaluated included serology, vireamia, clinical signs relevant to BTV8, body temperature.

Results:

Clinical monitoring and body temperature: No clinical signs which could be attributable specifically to BTV were observed in any of the animals. Both single and repeated vaccination, no matter the antigen dose, prevented the increase of temperature observed in the control animals. A peak of temperature above 40ºC appeared between 5-6 days after challenge, and lasted for 3-4 days in both control groups. This difference in temperature was statistically significant.

Viraemia: Prevention of viremia was observed in all animals of G1, G3 (animals vaccinated twice) and in G5 and G6 (animals vaccinated once). Protection against viremia in G2 and G4 was 98,8 % and 86,6 % respectively. Conversely, BTV nucleic acid was detected by RT-PCR consistently in blood of all control sheep (G7 and G8) from D2 to the end of the experiment.

Serology: All repeatedly vaccinated sheep were seropositive by competitive enzyme-linked immunosorbent assay (c-ELISA) three weeks after the second vaccination. In single vaccinated sheep, BTV-specific antibodies were detected in 95.2% of the animals eight days following the challenge. Regarding BTV-neutralising (VN) antibodies VN titers could be detected in 64.3% sheep after one vaccination. After the second vaccination, all sheep developed VN antibodies. Subsequently to the challenge, the VN titers increased for all vaccinated animals. In both vaccine regimes, the VN titres were significantly higher than those from control animals after the first vaccination and/or at the day of challenge and at day 8 post challenge, indicating a booster effect of the challenge in vaccinated animals.

Conclusions:

It was concluded that vaccinated sheep were protected from viremia associated to BTV challenge irrespective of the antigen dose. Furthermore, it has been shown that protection could be obtained with a single vaccination, even at the lowest antigen content, upon a subcutaneous challenge with a high dose of a virulent heterologous BTV-8.

Determination of the Vaccine Dose/Onset of protection in cattle

Efficacy of subcutaneous vaccination with two dose volumes of inactivated BTV serotype 8 in 6-15 week old susceptible calves

Objective

Efficacy (and general safety) of a repeated (2x) subcutaneous injection of different dose volumes of a production was evaluated in calves. To this aim the clinical reaction index, viraemia and the humoral response were assessed after challenge.

Animals

Calves negative for antibodies against BTV were selected for the study. The animals were distributed in 3 groups. The health status of the animals was assessed after each administration of the vaccine. Additionally, measurement of rectal T° was carried out on Day -2, on Day -1 and just before vaccination. The study was terminated 3 weeks after challenge. Animals were euthanised and submitted to post mortem examination of injection sites followed by microscopical examination of lesions.

Treatment schedule

Two vaccination schedules were evaluated. Group 1 was vaccinated on Day 0 and Day 21 with 1ml of the vaccine whereas Group 2 with 2ml on the same days as Group 1. Controls of Group 3 were injected with PBS at the same time as vaccination. All animals were challenged on Day 42. Test articles were administered by subcutaneous route. The study terminated 21 days after challenge (D63)

Post-vaccination follow-up

Blood samples to determine antibodies to BTV (ELISA and VN test) were collected just before each vaccination, at challenge and at established time points after challenge throughout the end of the study.

Post-challenge follow-up

Calves were monitored for rectal temperature and for the occurrence of clinical signs over the whole observation period. Blood samples were collected every two days from just before challenge until the end of the study.

Results

Validation of challenge: Fever scores were significantly higher in the controls than in the vaccinated animals during 4-14 days time period after challenge. Increase of body temperature to BTV was observed in control animals only. Viral RNA was detected (one exception only) in all blood samples collected from control animals.

Clinical monitoring: No clinical signs attributable to BTV were observed in any vaccinated animals. Between 4 and 14 days after challenge, the fever score was significantly higher in the controls than in the vaccinated animals.

Viraemia: Viral RNA load in the vaccinated groups was significantly reduced compared to the control group. There was no significant difference in viral RNA load between the two vaccinated groups (G1 and G2).

Serology: 33% of control calves were still negative on D54. BTV specific antibodies were detected in the vaccinated animals at the time of challenge and remained positive after challenge. The vaccinated animals started to develop VN antibodies from one week after challenge and the titre increased rapidly until the end of the study.

Conclusions:

This study supports the claim of reduction of viraemia in calves induced by the administration of the vaccine.

Determination of dose volumes for inactivated BTV serotype 8 vaccine in susceptible calves

Objective

Two dose volumes of Bovilis BTV8 were tested in order to determine the efficacy in cattle.

Animals

Two groups of 2-3 months old calves were vaccinated twice with an interval of three weeks with two dose volumes of Bovilis BTV8 (G1 and G2). An additional group of placebo (physiological saline solution) injected calves was included to serve as controls. All injections were given subcutaneously. Three weeks after the second vaccination all animals were challenged with virulent heterologous BTV-8 virus. Blood samples were collected from all calves at different time points throughout the experiment to monitor the presence of antibodies against Bluetongue virus. In addition, blood samples were taken every two days in the challenge phase in order to measure viraemia. From just before challenge until three weeks after, rectal temperatures were measured and the animals were observed for general clinical reactions and specific lesions at the mouth, nostrils, and feet.

Treatment schedule

The two vaccination schedules as described above were evaluated (i.e. Groups 1 and 2). A control group (G3) was also used.

Results

A significant difference between the control and the vaccinated groups was observed and there was no significant difference between the two Bovilis BTV8 vaccinated groups.

In both vaccinated groups a significant reduction of viraemia could be demonstrated compared to the viraemia in the control group.

Also in this experiment a rapid and significant increase in neutralizing antibody titres was demonstrated following challenge in both Bovilis BTV8 vaccinated groups, which indicated that the vaccination had resulted in a priming of the immune response. The increase in neutralizing antibody titres in both vaccinated groups was comparable.

Conclusions:

This study can support the claim of a reduction of viraemia in calves induced by the administration of the vaccine.

Dose response studies in sheep and cattle

The studies investigating the dose response using the optimised potency test were provided. In sheep vaccinated with a batch containing ¼ of the antigen content there was no evidence of viraemia. The results of a quarter (¼) dose were effectively the same as those achieved with a full dose when challenge was administered after 21 days.

In cattle vaccinated with a batch containing either a half (½) or ¼ of the antigen content the frequency and duration of viraemia appears insignificantly increased. Therefore, in the case of cattle whilst a ½ or ¼ dose is still efficacious (compared to unvaccinated controls), these batches showed a tendency to be not as efficacious as a full dose (when challenge was administered after 21 days). However, only a batch containing one eighth (1/8) of the antigen was significantly less effective as a normal batch of Bovilis BTV8.

With the aim to demonstrate how the level of reduction in viraemia may reflect the capability of the vaccine to interrupt virus transmission in cattle the applicant provided supportive evidence to demonstrate that the chance of infection of vector and subsequent animal is low to zero when the levels of viremia correspond to Cycle threshold (Ct) or Crossing Point (Cp) values of ≥ 30-32 (determined by rRT-PCR methods). Ct and Cp values are equivalent but determined based on a different type of software package.

Since in the efficacy studies different RT-PCR's were used, the applicant presented a case for having equivalent sensitivity. The methods have all been independently validated and have also been compared with each other for correlation. The level of virus required for transmission is based on published literature (Hamers et al 2009) and the Manual of diagnostic tests for vaccines for Terrestrial Animals 2004. These levels of virus have been linked to the applicants RT-PCR validation studies for limit of detection. An RT-PCR of ≥ 30-32 has been considered below the level permitting transmission.

In sheep vaccinated with 1 dose and ¼ dose the RT-PCR was seen to be at the upper limit of the test. In cattle there was one animal that received a full dose with an RT-PCR of just below 30 although there was also a calf which had two questionable (positive) RT-PCR results. These data questioned the level proposed as correlated with prevention of transmission (≥30-32 Cp/Ct). In general, as the dose reduces so the duration and frequency of animals with a positive RT-PCR increases, with an associated reduction in RT-PCR result (i.e. higher virus level). Most cattle receiving a full dose consistently have an RT-PCR of 46, which would confirm the meaningfulness of the lower RT-PCR results indicative of viraemia.

Based on these data and analysis the CVMP accepted that vaccination prevents viraemia in sheep and reduces viraemia in cattle.

Epidemiogical modelling to investigate the effect of vaccination in cattle.

Two models were provided to investigate the effect of vaccination on the risk of bluetongue to cattle. The two models were prepared by two independent expert groups involved in modelling BTV spread. Both models used as the basis the basic reproduction number (R_0) to estimate the number of additional cases which could arise from an initial case of BTV infection in cattle. If the R_0 is less than 1 this indicates that transmission will not occur.

Study designs:

The modelling was based on the most recent laboratory efficacy studies which looked at the dose-response and the link to potency in chickens described earlier (in sheep and in calves).

Model 1 appeared only to use data from the same cattle experiment whereas model 2 referred to both the cattle study and the sheep study.

In both models it was necessary to make a range of assumptions (for example, probability of transmission from vector to host, probability of transmission from host to vector, biting rate, time between blood meals, vector preference for cattle compared to sheep). The reliability of the models therefore was based significantly on the correct choice of parameters. These parameters were drawn from published literature.

<u>The two models produced similar results:</u>

Model 2 studied two scenarios, one which only used results below 30 Cp and one where all dubious RT-PCR results were included. In both scenarios the reproduction number (Ro) was found to be less than 1, indicating that introductions onto vaccinated farms would tend to die out.

Model 1 also applied the same two scenarios. This analysis concluded that the reproduction number would not always be below 1 but that vaccination did induce a substantial amount of reduction of transmission.

<u>Conclusions:</u>

As the review of the results of these models required highly specialist knowledge of modelling systems and their relation to Bluetongue the CVMP established an ad-hoc Scientific Advisory Group (SAG) composed by experts in the field of BT modelling epidemiology in order to provide an opinion to this specific issue. The ad-hoc SAG concluded that both mathematical transmission models were suitable to demonstrate relevance in control at the population level. Both models mimicked or incorporated relevant biological processes in their structures, according to current scientific knowledge. The experimental data presented in the dossier demonstrated a statistically significant reduction in both duration of viraemia and proportion of animals becoming viraemic. Both mathematical transmission models, building on these experimental data and simulating it at the population level, provided some evidence that the vaccine can reduce levels of transmission when compared to that in an unvaccinated population. However, this would be expected, simply, from the reduced, but present, levels of viraemia observed after challenge in cattle that was demonstrated in the experimental trials. It was also noted that the degree of transmission that will occur in a population vaccinated by this product would depend on several parameters, including the density of cattle and sheep and the ratio of competent *Culicoides* spp. vectors, the temperature and its daily fluctuation, the vaccine coverage and any change in efficacy of vaccination in the period following administration of vaccine. All of these parameters are subject to considerable variation and there is uncertainty in relation to how the effect of each should scale in the models. The model outputs reflected uncertainties in the true value of these parameters. This was also made clear in the papers by the modellers when they draw attention to their limitations. Neither model provided an absolute assurance of a lack of transmission in a vaccinated population. This is a consequence of the uncertainty in the parameterisation. Only Model 2 considered the transmission between herd.

From what is currently known, it was considered for both models that a conservative approach was taken in considering the likely efficacy of the vaccine at the population or community level as for example, data from diluted vaccine trials have been merged in with data from potent vaccines to provided estimates of vaccine efficacy and evidence existed for effectiveness of the vaccine in the European Community and thus the vaccine could contain an outbreak in vaccinated population.

On the basis of the above the CVMP agreed to recommend a reduced vireamia claim for cattle from 6 weeks of age with a clarification in section 4.6 of the SPC that the product does not prevent viraemia in cattle. The extent of this reduction has been shown by epidemiological modelling studies to be likely to reduce virus transmission to an extent that can limit the spread of an outbreak in a vaccinated population.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

Data were not provided on the impact of Maternally Derived Antibodies on vaccine efficacy in animals of any of the two target species but specific studies are stated to be planned by the applicant. A warning is included in the relevant section of SPC to reflect the above.

Duration of Immunity

Studies regarding a 6-month duration of immunity in sheep and cattle were presented. A reference batch (081169) was used in both studies. In order to demonstrate the level of protection achieved by Bovilis BTV8 against an experimental challenge with BTV8 carried out 6 months after vaccination in sheep and cattle was demonstrated.

Sheep

Six month duration of immunity study in lambs

BTV free and antibody negative conventional 4-8 weeks old Ripolesa lambs were vaccinated subcutaneously with Bovilis BTV8. Lambs were allotted to four treatment groups (G1 to G4). Animals of G1 were vaccinated once (D21) with 1 dose (1 ml) of the vaccine. Animals of G2 and G4 received two single dose vaccinations with an interval of 3 weeks (D0 and D21). The animals of G3 were injected twice (D0 and D21) with 1 ml of phosphate buffered saline solution as a placebo. Six months after D21 (D203), 8 randomly chosen animals of G1, G2 and G3 were subcutaneously injected with 10 ml of a BTV8 virus suspension At the same time point, animals in G4 were revaccinated with a single dose of the vaccine.

Follow up: From four days before challenge until 3 weeks after, rectal temperatures were measured in all challenged animals which were also monitored for the appearance of general clinical reactions and specific lesions at the mouth, nostrils and feet. Suitable blood samples were taken from all animals at selected time points in order to monitor the antibody response or to measure viraemia (this was assessed by validated RT-PCR and by virus isolation).

Results: The experimental challenge did not result in any clinical effects specifically related to BTV8 infection. There was no evidence of a statistical significant difference in mean rectal temperature profiles after challenge between G1 and G2. Both groups were however significantly different from the controls G3 where 6 out of 8 animals developed fever in the period between 3 and 14 days after challenge. At point of challenge (6 months post first injection) all vaccinated animals were tested positive for BTV-8 VN and ELISA antibodies. At this point all controls of G3 were still negative. After challenge, titres increased rapidly in all vaccinated groups, while controls needed more time to respond. Seven out of eight controls of G3 were positive in the ELISA 6 days after challenge. Two weeks after challenge no difference between the animals of G1 and G3 was observed anymore.

Seven out of eight controls of G3 showed viraemia 4 to 6 days after challenge. None of the vaccinates of G1 and G2 showed viraemia, neither as determined by PCR nor by virus isolation.

Cattle

Six month duration of immunity study in calves

In order to demonstrate the level of protection achieved by Bovilis BTV8 against an experimental challenge with BTV8 carried out 6 months after vaccination, BTV free and antibody negative conventional 8-12 weeks old dairy calves were vaccinated subcutaneously with Bovilis BTV8. Calves were allotted to four treatment groups. Animals of G1 and G4 were injected twice (D0 and D21) with 1 ml dose of the vaccine. Animals of G2 were injected twice (D0 and D21) with 2 ml dose of the vaccine. Animals of G3 were injected twice (D0 and D21) with 1 ml of physiological saline solution as a placebo.

Six months after the second vaccination eight randomly chosen animals of G1, G2 and G3 were subcutaneously injected with a BTV8 virus suspension. At the same time point, animals in G4 were revaccinated with a single dose of the vaccine.

Follow up: From two days before challenge until 3 weeks after, rectal temperatures were measured in all challenged animals which were also monitored for the appearance of general clinical reactions and specific lesions at the mouth, nostrils and feet. Suitable blood samples were taken from all animals at selected time points in order to monitor the antibody response or to measure viraemia (this was assessed by validated RT-PCR and by virus isolation).

Results: The experimental challenge did not result in any clinical effects specifically related to BTV8 infection. The mean rectal temperature profile showed no evidence of a statistically significant difference between G1 and G2, whereas both groups were significantly different from the controls of G3. Due to the fact that the rise in rectal temperature in controls occurred later than normally expected after challenge, and also due to the limited degree of this rise the relation between the rise in rectal temperature and the challenge was considered to be unlikely. From three weeks after the second vaccination low VN titres developed in all vaccinates of G1, G2 and G4. However, within two weeks after challenge high titres developed in all vaccinated animals. In the controls of G3 it took more than two weeks after challenge before all animals had developed VN titres against BTV.

All vaccinated animals developed ELISA titres against BTV within one week after re-vaccination. All control animals were tested negative until one week after challenge. Two weeks after challenge all controls of G3 were positive. The viraemia in the controls of G3, as determined by PCR, started to develop from day 4 post-challenge. Eight days post- challenge all controls were positive by virus isolation.

PCR-positive samples were found in three vaccinates from G1 and two from G2. Virus could be isolated from samples from one animal of each G1 and G2 during 10 days.

For both parameters, PCR-score and virus isolation score, the difference between vaccinated and control groups was statistically significant, but no significant difference between groups 1 and 2 was observed.

Conclusions:

Taking into account the expected level of protection induced by the vaccine (i.e. prevention of viraemia in sheep and reduction of viraemia in sheep, it can be concluded that overall evidence was provided for a 6 months duration of immunity in both sheep and cattle.

Prevention of Transplacental Transmission

No data were available to date to show the efficacy of the vaccine when used in pregnant animals.

Field Trials

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), field trials may be omitted. To supplement the numerous laboratory studies presented, six additional summary reports describing the outcome of field studies were made available (full list can be found in relevant section of the Safety Part). However, it is expected that the applicant will provide the full set of data and any further supportive information becoming available on the efficacy of the vaccine in the field.

Overall conclusion on efficacy

Sheep: Satisfactory data were provided of the efficacy of the vaccine to prevent viraemia with preparations containing low antigen payloads. Satisfactory information was also provided for the selection of the dose from studies in lambs of the minimum age. Study results supported a duration of immunity of 6 months. The onset of immunity is 3 weeks after completion of the basic vaccination course which consists of one single injection in sheep. No additional efficacy data were generated in pregnant animals of the target species.

Cattle: A reduction in viraemia was demonstrated while it could not be established that the level of reduction of viraemia is capable of completely blocking the transmission of infectious virus from vaccinated cattle. However on the basis of epidemiological modelling studies it was shown that the the extent of the observed reduction in viraemia was epidemiologicaly relevant and likely to reduce virus transmission to an extent that can limit the spread of an outbreak in a vaccinated cattle population. This was acceptable under the exceptional circumstances status of the application. Therefore a claim for reduced viraemia for cattle from 6 weeks of age was recommended by CVMP with a special warning at section 4.6 of the SPC that this reduction is not a prevention and the extent of this reduction as shown by epidemiological modelling studies is likely to reduce virus transmission to an extent that can limit the spread of an outbreak in a vaccinated population.

5. Benefit risk assessment

Introduction

Bovilis BTV8 is an inactivated vaccine prepared from a bluetongue serotype 8 strain isolated in 2006 from a cow located in the southern part of the Netherlands. The vaccine is inactivated with binary ethyleneimine and formulated to contain aluminium hydroxide and saponin as an adjuvant system. The product contains an active ingredient which is currently present in other products authorised within the EU by either the centralised route or through national provisions. The product is for a bluetongue vaccine and as such is being considered for an application for authorisation under exceptional circumstances.

Benefit assessment

Direct therapeutic benefit

Bovilis BTV8 is a vaccine containing inactivated serotype 8 bluetongue virus antigen combined with an adjuvant intended to induce an immune response in sheep and cattle, with the aim of eliciting protective levels of immunity capable of controlling BTV8 infection.

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.

Clinical trials demonstrated that the product is capable of inducing an immune response which reduces viraemia in both sheep and cattle. The effect in sheep would be to prevent transmission. The reduction in cattle would not prevent transmission.

Additional benefits

Bovilis BTV8 is a standard inactivated vaccine and as such fits with accepted vaccination practices in the field.

Duration of immunity of 6 months has been demonstrated in sheep. A reduction in viraemia was seen in cattle challenged 6 months after vaccination. The duration of immunity is expected to cover a risk period during which transmission would be most likely to occur.

Vaccination has been shown to be safe for use during pregnancy in both sheep and cattle, 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.

Risk assessment

Main potential risks:

- For sheep and cattle there is a risk of a slight rise in temperature and temporary swellings at the injection site following vaccination. These swellings typically last for over 3 weeks in sheep and may last in excess of over 6 weeks in cattle.
- In cattle the reduction in viraemia would not be expected to prevent transmission of BTV8 from vaccinated cattle consequently there is a risk of spread to other animals.
- 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 a 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 over failure to observe an MRL. The product contains components found in other marketed products, therefore the risk is no greater than already exists.

Specific potential risks, according to product type and application:

- Vaccination of cattle does not prevent transmission of wild type virus, therefore, blocking of spread of the disease cannot be ensured. This is an important objective of BTV vaccination campaigns.
- 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.

Risk management or mitigation measures

- Appropriate warnings have been placed in the SPC to warn of the potential risks to the target animal, the end user and the environment.
- 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 II assessment.

Evaluation of the benefit risk balance

The product has been shown to have a positive benefit risk balance. The product has been shown to be efficacious for the indication prevention of viraemia in sheep and reduction of viraemia in cattle. In addition, it has been demonstrated that the level of reduction of viraemia in cattle is capable of reducing transmission of virus.

The formulation and manufacture of Bovilis BTV8 are described and specifications are supported. The applicant is able to detect sub-potent batches thereby ensuring that 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. Withdrawal period is zero days.

Conclusion on benefit risk balance

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

- Bluetongue disease is spread by insect vectors and therefore presents particular challenges in terms of control due to an inability to prevent transmission from infected animals other than through insect control combined with reducing or preventing viraemia (virus in the blood) in susceptible animals by means of vaccination.
- Bluetongue disease is epizootic in nature and has the potential to result in high morbidity and mortality in susceptible populations, particularly of sheep.
- There is a remaining epidemiological risk from Bluetongue serotype 8 for European sheep and cattle populations, that constitutes an objective need to have authorised products available for use in the coming months.
- That consequently any delay should be avoided where possible in making available safe and effective vaccines that have been demonstrated to be in compliance with the CVMP guideline on Minimum Data Requirements for an Authorisation Under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/IWP/220193/2008).

Conclusion

On the basis of the above 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. The authorisation of the product will be subjected to annual re-assessment in order to recommend whether the authorisation should be continued or not. In addition, the commitments undertaken by the applicant must be fulfilled, in order for the authorisation to revert to normal status, i.e. no longer exceptional and subject to annual review.

Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Directive 2001/82/EEC.