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Veterinary Medicines and Product Data Management

Scientific discussion

This module reflects the initial scientific discussion for the approval of BLUEVAC BTV8 (as published in April 2011). For information on changes after this date please refer to module 8.

1. Summary of the dossier

BLUEVAC BTV8 is an inactivated vaccine presented as a suspension for subcutaneous (SC) injection.

BLUEVAC BTV8 is intended for the active immunisation of sheep and cattle from 2.5 months of age for the prevention of viraemia in sheep and cattle and to reduce clinical signs caused by bluetongue virus (BTV), serotype 8 in cattle. The active substance of BLUEVAC BTV8 is the inactivated bluetongue virus serotype 8. The vaccine dose for sheep is 2 ml and for cattle 4 ml. Primary vaccination in sheep includes two doses of 2 ml subcutaneously with a 3 week interval; in cattle two injections of 4 ml are administered with an interval of approximately 3 weeks.

BLUEVAC BTV8 was eligible for the centralised procedure under Article 3(2) of Regulation (EC) No 726/2004 as it is an immunological veterinary product for the treatment of animal disease subject to community prophylactic measures.

BLUEVAC BTV8 stimulates active immunity in sheep and cattle against bluetongue virus serotype 8, resulting in prevention of viraemia in sheep and cattle and reduction of clinical signs in cattle. Onset of immunity is 20 days and 31 days, respectively after the second dose of the primary vaccination course. The duration of immunity is one year for both sheep and cattle.

BTV can cause intense disease outbreaks in sheep. Fever is the most usual but not invariable clinical sign. If fever occurs sheep first got pyrexia 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 and 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 bluetongue is less common in cattle, some clinical signs have appeared in recent epizootics in Northern West Europe caused by the BTV8 serotype. The most prominent lesions in BTV8 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

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outbreaks due to serotype 8 occurred in the Netherlands, in Belgium, Germany, Luxemburg, France and in the UK. It is considered likely that the disease will remain in Europe for the next few years creating an endemic situation.

The dossier was reviewed in line with the provisions of Article 39(7) of Regulation (EC) No 726/2004 for an authorisation under exceptional circumstances and the recommendations of the CVMP 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

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

Names of ingredients	Quantity per 1 ml	Function	Reference to standards
Active Substance			
Bluetongue virus, serotype 8, inactivated	$10^{6.5}$ $CCID_{50}$ *	Active ingredient	In-house specification
Excipients			
Aluminium hydroxide	6 mg	Adjuvant	Ph. Eur. monograph 1664
Purified saponin (Quil A)	0.05 mg	Adjuvant	In-house specification
Thiomersal	0.1 mg	Preservative	Ph. Eur. monograph 1625
Phosphate-buffered saline, pH 7.6			
Potassium dihydrogen phosphate		Diluent	Ph. Eur. monograph 920
Disodium phosphate dihydrate			Ph. Eur. monograph 602
Sodium chloride			Ph. Eur. monograph 193
Water for injection			Ph. Eur. monograph 169

* equivalent to the titre prior inactivation

Container

BLUEVAC BTV8 is presented in boxes containing high density polyethylene (HDPE) bottles of 52, 100 or 252 ml, closed with rubber stopper and aluminium cap.

HDPE bottles and the stoppers made of bromobutyl rubber were manufactured in accordance with the relevant European Pharmacopeia (Ph. Eur) monographs.

Development Pharmaceutics

BLUEVAC BTV8 is an inactivated vaccine presented as a suspension for injection.

The BTV8 strain is the active ingredient (vaccine antigen) and was isolated from an outbreak that occurred in Belgium in 2006. The antigen content is fixed at $10^{6.5}$ $CCID_{50}$ per dose of 1 ml.

The production process of the active ingredient (BTV8) as well as the finished product followed a standard procedure.

The BTV8 strain is propagated on BHK21 cells (permanent Baby Hamster Kidney cell line). This cell line is well-known and used for many years with good experiences in other vaccines authorised in Europe. The BHK-21 cells which are used as the host system and the BTV8 virus which is used as the active ingredient (vaccine antigen) are handled in seed-lot systems using Master and Working Seeds. The vaccine virus is inactivated by using binary ethyleneimine (BEI) as the inactivation agent. The use of BEI was based on good experience in the production of vaccines.

Aluminium hydroxide and purified saponin are added as components of the adjuvant. The use of both excipients was based on their well-known properties. Both substances are used in other inactivated vaccines licensed in Europe.

The vaccine is a multi-dose product contains thiomersal as preservative which is widely used in veterinary vaccines. The efficacy of this preservative has been established in accordance with the Ph. Eur. requirements.

Composition of the batches used in the clinical trials

Data were provided for batches used in safety and efficacy trials. These vaccine batches were produced according to the outline of production and they were considered appropriate for use in the different studies.

Validation studies

A number of validation studies were submitted as part of the validation of the manufacturing process and the results were considered acceptable.

Method of manufacture

A detailed flow chart of the manufacturing steps including reference also to the controls carried out during the manufacturing process and on the finished product was provided and was considered satisfactory.

In summary for virus mass cultivation, the vaccine virus is propagated in BHK-21 cell. After clarification, the virus is inactivated using BEI, the inactivated virus is purified and concentrated, and the BEI is neutralised. Vaccine bulks are prepared by blending the antigen bulks with all other components to a vaccine suspension. The volume is according to the amount of vaccine bulk to be prepared and the viral titres of the inactivated antigen bulks.

For preparation of the finished product, the vaccine bulks are filled into sterile vials which are then closed with rubber stoppers and aluminium caps, labelled, packaged and stored.

Control of starting materials

Listed in a Pharmacopoeia

Details were provided for the following substances, and compliance with the relevant Ph. Eur. monographs was established with the provision of the corresponding certificates of analyses:

Starting Material:

- Purified water
- Water for injection
- Sodium bicarbonate
- Sodium carbonate
- Potassium chloride
- Sodium chloride
- Disodium edetate
- Potassium dihydrogen phosphate
- Disodium phosphate dihydrate
- Aluminium hydroxide
- Sodium hydroxide
- Thiomersal
- Sodium thiosulphate

The provided information was acceptable.

Not listed in a Pharmacopoeia

Starting materials of biological origin

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

Starting Material

- Bluetongue virus, serotype 8
- BHK-21 hamster kidney cell line (SA) – suspension
- BHK-21 (clone 13) hamster kidney cell line – monolayer
- Bovine serum albumin
- Sterile adult bovine serum
- Foetal bovine serum
- Trypsin
- Tryptone
- Purified saponin (Quil A)

BHK-21 hamster kidney cell line (SA and clone 13)

The cell line BHK-21 SA is used for virus propagation. The cells have the capacity to grow in suspension (fermenter) rather than as monolayer. From the historical point of view, *Stocker and MacPherson* (1962) were able to culture baby hamster kidney cells, from which originated the BHK-21 line, clone 13. The cell line was then adapted for growth in suspension by *Capstick et al* (1962).

The cell line BHK-21 clone 13 is used to prepare the virus seeds for BTV8. The cell line has fibroblast morphology and the capacity to grow as monolayer.

A summary on the preparation and testing of the Master Cell Seed (MCS) of the BHK-21 cell line was presented. Detailed protocols and reports on the examination of the MCS were submitted.

The test on exclusion of bacteria and fungi (sterility) resulted in no growth of contaminants in MCS. The test for exclusion of mycoplasma on MCS resulted in no growth of contaminants.

For the exclusions of extraneous viruses on the MCS controls were performed using direct and indirect test methods according to the relevant guidelines; neither cytopathic effect (CPE) nor hemadsorbing effects were observed.

Tests in other cell cultures for specified viruses were also carried out and no such viruses were detected. Further detection tests on bovine, ovine and the species of the origin of the cell line specified bacteria and viruses, were performed. The Master Cell Bank (MCB) was also controlled for identification of species and karyology.

In the Working Cell Bank (WCB) the same controls as for MCB were carried out with the exception of the identification of species and karyology.

Bluetongue virus, serotype 8

Master Seed Virus:
The origin and history of the virus strain was adequately explained. The virus was isolated from a sheep blood sample was passaged in chicken embryos and in BHK-21 cells. A summary on the origin, history and testing of the Master Seed Virus (MSV) was presented. Controls to demonstrate bacterial, fungal and mycoplasma sterility, absence of specific extraneous agents, the identity of the virus strain, virus titre and residual moisture were performed. Results were acceptable.

Working Seed Virus:
The Working Seed Virus (WSV) was prepared from the MSV.

Controls to demonstrate bacterial, fungal and mycoplasma sterility, absence of specific extraneous agents, the identity of the virus strain, virus titre and residual moisture were performed. Results were acceptable.

The WSV passage 1 was prepared from the WSV to start the viral antigen production following on passage.

The following controls and tests according to relevant requirements have been carried out on the WSV passage 1 with satisfying results: bacterial and fungal contamination, mycoplasma contamination, viral content.

In-house preparation of media:
Description of constituents, method of preparation, including sterilization is provided for in-house media: e.g. cell growth medium and virus maintenance medium and solutions.

Overall the information supplied regarding the starting materials (active substance, excipients) was considered acceptable. The documentation gave necessary information regarding their function, species origin and treatment before use. Certificates or the compliance with Ph. Eur., where relevant, were provided.

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

An assessment of the starting materials was conducted taking into account the "Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" (EMEA/410/01-Rev.2), the "Position Paper on the assessment of the risk of transmission of animal spongiform encephalopathy agents via master seed materials used in the production of veterinary vaccines" (EMEA/CVMP/019/01) and the "Position Paper on the risk

assessment of the use of starting materials of ruminant origin in veterinary medicinal products intended for use in ruminant species" (EMEA/CVMP/121/01).

The starting materials of animal origin are listed in the table below with information on their origin and on the way the applicant demonstrated compliance:

Starting materials of animal origin	Demonstration of TSE compliance	Origin/tissue category*	Country of origin/ GBR level
BHK-21 (clone 13) hamster kidney cell line - monolayer	Statement and assessment	-	
BHK-21 hamster kidney cell line (SA) – suspension	Statement and assessment	-	
Bluetongue virus, serotype 08	Statement and assessment	-	
Bovine serum albumin	Statement	Blood/B	New Zealand/I
Adult bovine serum	Statement	Blood/B	Australia/I New Zealand/I
Foetal bovine serum	Statement	Blood/B	Australia/I New Zealand/I
Tryptone (bovine)	Statement	Milk/C	New Zealand/I
Trypsin (bovine, porcine)	Statement	Milk/C	USA/III

*Category B "Lower infectivity tissue" and Category C "Tissue with no infectivity"

Confirmation is given that no material from GBR III level countries and sera from New Zealand and Australia only will be used.

Bovine serum:

Assurance that the donor animals comply with the regulations concerning TSE including specific and adequate EDQM certificates of suitability were provided.

Seed materials and other starting material of animal origin relevant for the transmission of TSE complied with the 'Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products' (EMEA/410/01-Rev.2) and the corresponding Ph. Eur. monographs.

Overall 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. It was therefore concluded by CVMP that a risk of transmission of animal spongiform encephalopathy agent of BLUEVAC BTV8 can be excluded.

In conclusion the starting materials of animal origin used in the production of the final product comply with the relevant regulatory requirements of the Commission Directive 1999/104/EEC and the TSE Note for Guidance (EMEA/410/01-Rev.2).

Control tests during production

A detailed description was provided of the in process test controls during production.

The in-process tests performed during production of BTV8 antigen are the following:

- Sterility and purity at intermediate stages

- Cell count at intermediate stages
- Observation of cytopathic effect
- Complete inactivation
- Virus titration at intermediate stages
- Quantification of the antigen: The establishment and validation of a suitable method for the quantification of BTV8 antigen after inactivation at formulation is in progress.
- Residual sodium thiosulphate

The general characteristics, including validation where relevant, of these methods were supplied together with all necessary information. The results of the control testing carried out on three consecutive batches of BTV8 antigen were provided and were found compliant with the established specifications.

Control tests on the finished product

A detailed description was provided of the test controls performed on the finished product. The methods, frequency and pass criteria for the tests were provided in detail and were found to be acceptable.

The following tests have to be performed on each vaccine bulk:

- Appearance
- Test for pH
- Thiomersal content
- Saponin content: The establishment and validation of a suitable method is in progress
- Aluminium hydroxide content
- Complete inactivation
- Sterility

The following tests have to be performed on each finished product:

- Appearance
- Filling volume
- Sterility
- Identification
- Biological activity (potency)
- Safety

The general characteristics of these methods, including validation where relevant, were supplied together with the necessary information. Data were provided in order to demonstrate that robust and consistent batches of the vaccine under application will be produced. Results of testing have been provided for consecutive batches of BTV8 antigen and consecutive batches of the vaccine; all specifications were met.

Batch to batch consistency

Batch to batch consistency was shown as the applicant included results from three final batches of the current vaccine which were satisfactory in relation to production consistency.

Stability

A stability study to support the stability of the inactivated antigen was initiated.

Information from the ongoing stability study in the finished product from three batches produced in line with the described manufacturing practices and tested up to 27 months was provided. Activity results were obtained with the former serology potency test.

As stated in the applicable CVMP guideline on Minimum Data Requirements for an Authorisation under Exceptional Circumstances for Vaccines for Emergency Use Against Bluetongue (EMEA/CVMP/IWP/220193/2008) a maximum shelf life of 12 months may be granted in the absence of data. The provisional shelf life of 12 months could also be justified on the strength of the past specifications. Due to the need to provide authorised vaccines against BTV serotype 8 as soon as possible and the considerable time required in order to complete all stability studies in line with normal requirements the CVMP exceptionally accepted the current limited data with a view for the applicant to provide the remaining information as soon as available.

OVERALL CONCLUSION ON QUALITY

At present, with the data and clarification provided by the manufacturer, the quality profile of BLUEVAC BTV8 can be considered as 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 a antigen content of $10^{6.5}$ $CCID_{50}$/ml was shown to be efficacious in both sheep and cattle and even a batch with a lower titre showed to be still efficacious in both sheep and also cattle,
- the implementation of virus quantification at formulation step is in progress,
- each batch will be released on the basis of a challenge model on sheep,
- the production process allows production of consistent batches,
- current limited data on stability results can be exceptionally accepted due to the need to provide authorised vaccines against bluetongue serotype 8 and the considerable time required in order to complete all stability studies in line with normal requirements the CVMP with a view for the applicant to provide the remaining information as soon as available,
- the provision of information regarding the following remaining outstanding issues on quality are part of the applicant's specific obligations and relate to: a) In process control tests carried out on at least 3 vaccine antigen batches of different sizes, produced within the range of 250-5200 litres b) Final data supporting the 12 months storage time at 2 °C - 8 °C of vaccine antigen(s) c) Validation data of the antigen quantification at formulation stage d) Full set of data, according to the reported timelines, in order to demonstrate the claimed stability of finished product e) A validated test to quantify the saponin content in the finished product is awaited

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

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

3. Safety assessment and residues

BLUEVAC BTV8 is indicated for the active immunisation of sheep to prevent viraemia and to reduce clinical signs caused by BTV serotype 8 and for the active immunisation of cattle to prevent viraemia caused by BTV serotype 8. For sheep, one dose of the vaccine contains 2 ml. For cattle, one dose of the vaccine contains 4 ml. The recommended vaccination schedule is 2 doses of 2 ml for sheep and 2 doses of 4 ml for cattle at an interval of 3 weeks.

The safety studies were carried out in the target species cattle and sheep at the minimum age of 2.5 months and in pregnant animals. The vaccine batches that were used were representative for production and in accordance with the manufacturing process described. To support the safety profile additional studies were presented conducted from other bluetongue vaccines produced by the applicant that contained different serotypes such as 1, 1 - 4 and 4. These vaccines were of identical qualitative and quantitative composition as the company's BTV8 vaccine except for the serotypes included.

A. Safety assessment

Laboratory tests

The trials were performed in accordance with Directive 2001/82/EC as amended. Confirmation on the GLP compliance was given. 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) was taken into consideration.

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

SHEEP

Safety (and efficacy study) of the administration of one single dose of BLUEVAC BTV8 to sheep

The purpose of this study was to assess the safety (and efficacy) in sheep of minimum age (2.5 months) after subcutaneous administration of one single dose. BTV free and seronegative lambs were randomized in two groups. One group was vaccinated with one single dose according to the vaccination schedule. The second group was left as controls and received a placebo.

No general systemic reactions were observed except for a slight transient increase in temperature in several animals Approximately 14 days after the vaccination, serum-neutralising (SN) titres were detectable in 1/5 animals only. At that time, an anti-virus protein 7 (VP7) antibody titre was detectable at a low level in the serum pool.

Safety (and efficacy) study of a single dose and a repeated dose of BLUEVAC BTV8 in sheep

The purpose of this study was to assess the safety (and efficacy) in sheep of minimum age (2.5 months) after subcutaneous administration of a single dose, followed by the administration of a second dose.

BTV free and seronegative sheep were randomised in two groups. One group was vaccinated with two doses according to the vaccination schedule. The second group was left as control and received a placebo.

With regard to the development of adverse reactions, only in 2 animals the formation of a small nodule of 0.5 cm was observed at 14 days after the first inoculation.

An increase in temperature was observed in the majority of animals 4 hours after the vaccination. Increased temperature was temporarily observed in some animals ranging until the end of the 14-days observation period. Whereas only 20% of animals showed an increase in body temperature 4 hours after the administration of the repeated dose, all vaccinates developed a temperature increase the day after vaccination. Increased temperature was temporarily observed in one or two animals on day 2, 3, and 21 after the second vaccination.

Anti-VP7 ELISA antibody titres could be determined 21 days after the first vaccination. Both anti-VP7 ELISA and SN antibody titres were detectable 14 days after the second vaccination.

Overall conclusions from single and repeated dose studies in sheep:

The vaccine was well tolerated in sheep of minimum age recommended for vaccination. Within two hours after vaccination, no symptom of shock, indicating any abnormality, ever occurred. Adverse reactions were limited to an occasional transient increase in body temperature, whilst in very rare cases a febrile reaction occurred. Several times temporary local reactions were observed at the injection site in the form of a normally painless nodule of 0.5 to 1 cm after administration of the regular dose of 2 ml which disappeared until the end of the observation period.

CATTLE

Safety (and efficacy) study on the administration of a single dose and a repeated dose of BLUEVAC-8 in cattle

The purpose of this study was to assess the safety (and efficacy) in cattle of minimum age (2.5 months) after subcutaneous administration of a single dose, followed by the administration of a second dose. Two batches containing different antigen concentrations were used. Here, only the safety relevant data are considered.

BTV free and seronegative calves were included. Three groups were formed. Two groups were vaccinated subcutaneously and the third group remained as control. Vaccination followed the proposed vaccination schedule. The controls received a subcutaneous application of phosphate buffer solution (PBS).

A slight increase in temperature was observed in 20% of the animals on four consecutive measurements after vaccination. This percentage belonged to the group that received the higher antigen concentration. 60% of the animals showed an increase in body temperature 4 hours after the second vaccination. Specific SN antibody titres were detected only at very low levels in 60% of the vaccinated animals on the day of the second vaccination but an increase of the titres was observed after the second vaccination Also an increase of the ELISA antibody titres was recorded after the first vaccination and after the second vaccination. The controls remained negative.

Conclusions:

After vaccination a transient increase of body temperature occurred of both groups. However this was acceptable and reflected in the Summary of Product Specifications (SPC) of the product. Thus the safety of the vaccine was considered as demonstrated in calves of young age when injected twice with BLUEVAC BTV8 with a 4ml dose.

Safety (and efficacy) study on the administration of a single dose (2 ml) and a repeated dose (2 ml) of BLUEVAC-8 in cattle

The purpose of this study was to assess the safety (and efficacy) in cattle of minimum age after subcutaneous administration of a single dose, followed by the administration of a second dose. But

deviating from the regular vaccination protocol, one dose consisted of 2 ml instead of 4 ml. Here, only the safety relevant data are considered.

BTV free and seronegative calves aged 2 months were included. One group was vaccinated and the other group remained as control. Vaccination followed the proposed vaccination schedule but with 2 ml only. The controls received a subcutaneous application of PBS.

A slight increase in temperature was observed in 16.6% of the animals on the day after the first vaccination followed by fever on day 4 post vaccination. The statistical evaluation did not reveal any statistically relevant difference between vaccinated and unvaccinated animals. After the administration of a repeated dose 50% of the vaccinated animals showed a very low increase in body temperature 4 hours after vaccination and on days 1, 2, 3 after the vaccination.

Specific SN antibody titres were detected in one calf of the vaccinated animals on the day of the second vaccination. 14 days after the second vaccination the SN antibody titres showed a significant increase. As regards the anti-ELISA antibody titres, the vaccinated animals showed a high titre not before 14 days after the second vaccination. The controls remained negative.

Conclusions: A transient increase of body temperature occurs after vaccination; however this was considered acceptable and thus the safety of the vaccine was considered as demonstrated in calves of young age when injected twice with a 2 ml dose. It was noted however that this was not the recommended dose.

Overall conclusions from single and repeated dose studies in cattle:

The vaccine was well tolerated in cattle of minimum age recommended for vaccination. Within two hours after vaccination, no symptom of shock, indicating any abnormality, ever occurred. Adverse reactions were limited to an occasional transient increase in body temperature, whilst in very rare cases a febrile reaction occurred. Several times temporary local reactions were observed at the injection site in the form of a normally painless nodule of 0.5 to 3 cm after administration of the regular dose of 4 ml which disappeared until the end of the observation period. The size of nodule increased after administration of the overdose.

Safety of one administration of an overdose

SHEEP

Safety study on the administration of an overdose and a repeated dose of BLUEVAC-8 in sheep

The purpose of this study was to assess the safety of subcutaneous administration of an overdose in sheep of minimum age (2.5 months) followed by the administration of a single dose.

The first group of BTV seronegative sheep were vaccinated twice in the neck according to the vaccination schedule at a 21-days-interval. A second group were left as controls and received a subcutaneous application of placebo.

A slight local transient reaction of 0.5 cm was observed in 20% animals 7 days after the repeated dose. The majority of sheep already showed elevated temperatures on the day of vaccination. Four hours after vaccination 87% of sheep showed a febrile reaction. During the following measurements the temperature remained elevated in approximately half of the vaccinated animals. Even the controls partly showed an increase in temperature. On the day of the administration of the repeated dose 50% of vaccinates already showed increased temperature. A further increase was observed within the first 24 hours after vaccination. The temperatures decreased until day 4 post vaccination in the majority of vaccinates.

Serum neutralising titres were not detectable until 14 days after the second vaccination whereas ELISA antibody titres were detectable at a low level already on the day of the second vaccination which clearly increased afterwards. The controls remained negative.

Conclusions: Following the administration of an overdose, at least an increase in body temperature was evident. Since the animals already showed an elevated temperature before vaccination, it was difficult to estimate if fever would have developed in case the animals would have had no elevated temperatures. However, a transient increase in body temperature occurred after each vaccination. In addition, minimal local reactions were observed in some sheep. The above were considered acceptable for an overdose study.

To support the findings of the overdose safety study, an additional study was carried out in sheep using a BLUEVAC BTV vaccine that contained serotypes 1 and 4 in oil adjuvant (which is not relevant for the vaccine under application). The results however were comparable to those achieved after the administration of BLUEVAC BTV8.

Safety study on the subcutaneous administration of an overdose in BTV free and seronegative three to five years old ewes in the early stage of pregnancy

The purpose of this study was to assess the safety of the subcutaneous administration of an overdose in BTV-seronegative three to five-years-old ewes in the early stage of pregnancy.

A group of pregnant sheep were vaccinated with a double dose (volume). A second group of pregnant sheep were left as controls and received a subcutaneous application of placebo.

The animals were observed for a period of 14 days after each vaccination and subsequently until parturition. Special care was taken within the first two hours after vaccination with regard to any symptom of shock. Monitoring after parturition was implemented and offspring was observed until 15 days after birth.

The pregnant sheep remained in good health after vaccination. No increase in body temperature occurred. Only a slight swelling could be observed at the injection site (A nodule < 1 cm developed at the injection site of each vaccinated animal as well as in each of the controls. The nodules disappeared in both the group of vaccinates and the group of controls until day 14 post vaccination). Parturitions proceeded without problems. The offspring were healthy with no health issues. The data obtained should be regarded as sufficient for the corresponding note in the SPC.

Conclusions: The data obtained were regarded as sufficient to support the safety of an overdose in pregnant sheep especially as another study was performed in ewes at the early and late stage of pregnancy.

CATTLE

Safety study of the administration of an overdose and a repeated dose of BLUEVAC-8 to cattle

The purpose of this study was to assess the safety of subcutaneous administration of an overdose in cattle of minimum recommended age (2.5 months) followed by the administration of a single dose.

A group of BTV free and seronegative calves were vaccinated twice according to the vaccination schedule at a 21-days interval but the first dose was of double volume. A group was left as controls and received a subcutaneous application of placebo.

The animals were observed for a period of 21 days after each vaccination.

The double dose application of BLUEVAC BTV8 resulted in a larger local reaction than the administration of the normal dose. However, any local reaction disappeared until the end of the

observation period of 21 days. Also the following repeated single dose revealed the typical swellings. Unexpectedly, not even a slight increase in body temperature was noticeable after the administration of the overdose whereas a negligible body temperature increase was observed after the repeated single dose in some animals.

Conclusions: An overdose of the vaccine appeared to be tolerated well in cattle supporting the safety of the product.

Examination of reproductive performance

SHEEP

In addition to the reproductive study in sheep cited above (overdose in ewes at early stage of pregnancy), a further study was carried out to assess the safety of BLUEVAC BTV8 after subcutaneous administration of a single and a repeated dose in 3 to 6 years old pregnant ewes, at the early or late stage of pregnancy.

Safety (and efficacy) study on the administration of a single dose and a repeated dose of BLUEVAC BTV8 in pregnant ewes

The purpose of this study was to assess the safety of BLUEVAC BTV8 after subcutaneous administration of a single and a repeated dose in 3 to 6 years old pregnant ewes, at the early or late stage of pregnancy.

BTV free and seronegative 3 to 6 years old ewes were included in this trial and subdivided into two vaccinated groups, according to their stage of pregnancy. Another group of sheep were kept as controls belonging either to the group of early or late stage of pregnancy. The controls received a subcutaneous application of placebo.

With regard to the development of any shock symptom after vaccination, special attention was paid during the first two hours after each vaccination.

The animals were observed for a period of 14 days after each vaccination and subsequently until parturition.

Monitoring after parturition was implemented and offspring was observed until 15 days after birth.

No symptoms of shock were observed during the two hours after the overdose administration. No serious local or systemic reaction occurred during the 14-days observation period. Several animals (irrespective of the group they belonged to) developed a nodule at the injection site (< 1 cm) after the 1st and 2nd vaccine application which disappeared within 4 days after vaccination at the latest. Since the controls also showed a local reaction, these reactions are probably caused by the injection technique. No increase in body temperature was recorded in any of the groups.

As regards pregnancy and birth, no abnormality occurred. Neither abortions nor teratogenic effects were detected. Litter size per ewe and lamb weight was normal with a mean value of 4 kg per lamb. Only one of the twins born in the group of early pregnancy died 15 days after birth. This is relatively common in indigenous Mediterranean breeds raised by extensive farming methods and was not related to the vaccination. The statistical analysis of the data in terms of stillbirths, number of births and death of one of the lambs in a twin parturition showed that there were no significant differences between the groups for these factors (Fisher's exact test).

Conclusions: Sheep in the early and late stage of gestation were vaccinated twice at an interval of 21 days with a normal dose of 2 ml of BLUEVAC BTV8. The pregnant sheep remained in good health after vaccination. No increase in body temperature occurred. Only a slight swelling could be observed at the

injection site. Parturitions proceeded without problems. The offspring were healthy. The death of one of the twins within the 15-days observation period was sufficiently explained. Thus, the data obtained were regarded as sufficient to support the safety of the vaccine in pregnant ewes in the early and late stages of pregnancy.

CATTLE

A report of a field trial was provided containing data on the examination of the reproductive performance of pregnant cows after the administration of one dose (4 ml) and a repeated dose (4 ml) of BLUEVAC-1. The composition of this vaccine was identical to that of BLUEVAC BTV8 in terms of adjuvants, preservative and other excipients, except for the serotype of the antigen vaccine.

Data were collected on three farms in Spain, as part of the mandatory vaccination programme against bluetongue serotype 1 of the Ministry of Agriculture, Fisheries and Food (2008).

Field trial on the examination of the reproductive performance in pregnant cows after the administration of one dose (4 ml) and a repeated dose (4 ml) of BLUEVAC-1

BTV seronegative dairy and beef cows were included in this trial. They were in the first, second and third trimester of pregnancy. The age of animals ranged between 1 and 18 years with a mean of 3 to 4 years.

The pregnant animals were examined for adverse reactions throughout the entire observation period: adverse effects on pregnancy or offspring were recorded. From parturition until 15 days afterwards, all animals born were observed in view of their vitality, weight at birth, number of stillbirths and survival rates at day 15 post parturition.

The diameter of any vaccination nodule developed after the first or second vaccine injection was recorded.

Data of cows vaccinated twice at an interval of 21 days in the last third of gestation with a BTV serotype 1 containing vaccine of CZ Veterinaria (CZV) were provided. The data referred to animals from the farm which presented the highest number of females in the last third of pregnancy at the time of the vaccination. No adverse reactions were observed in any of the animals. All the pregnant cows remained in good health after vaccination. Parturitions proceeded without problems. All animals were born normal and with the correct weight (> 30 kg), with good suckling reflexes. No apathy or anorexia occurred. The calves showed normal behaviour and a 15-day survival rate of 100%.

On this farm, an abortion at the 5th month of pregnancy was detected, 33 days after administration of the second dose of BLUEVAC-1. In the female that aborted, no significant infectious agent was isolated apart from the usual vaginal flora. No signs linked this abortion to the vaccination.

Conclusions: The data obtained were considered supportive of the product's safety in pregnant cows. Considering that 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) allows extrapolation of safety conclusions from vaccines with similar composition it was concluded that the safety of BLUEVAC BTV8 in pregnant cows has been established in this study.

No safety data were available in breeding males (neither cattle nor sheep). A corresponding note was introduced into the wording of SPC and package insert.

Examination of immunological functions

The examination of the immunological functions as required by Directive 2001/82/EC, as amended was discussed on the basis of the efficacy studies. However, further studies on immunological functions were not conducted since the vaccine is not expected to affect negatively the immune response of the vaccinated animal, as it is an inactivated vaccine for which the adjuvant has been shown to be safe. This approach was acceptable.

Ruminants infected with bluetongue virus develop a wide variety of protective antiviral responses that include interferon production and virus-specific cellular and humoral responses. Regarding the humoral response, the virus protein 2 (VP2) is the main determining factor of the serotype specificity and contains neutralising epitopes and hemagglutination sites. This protein is found on the outer capsid of the virus. The icosahedral internal nucleus of the virus contains the VP7 protein, which is apparently common to all BTV strains and serotypes. VP7 antibodies can be detected by ELISA tests. In this context, the immune response was studied in terms of serotype-specific neutralising antibodies and anti-VP7 antibodies which are common to all groups. At the time of second vaccination, SN antibodies are scarcely detectable, anti-VP7 antibodies only at a very low level. A clear increase of both sero-neutralising and anti-VP7 antibody titre is evident after the second vaccination.

Interactions

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

Field studies

Field trial in Germany (safety)

A field trial with BLUEVAC BTV8 was conducted in Germany with the collaboration of the Federal Research Institute for Animal Health (Friedrich-Loeffler Institute). The safety and efficacy profile of several BTV vaccines were assessed prior to the extensive use in the German field in 2008. With regard to the safety aspects of the vaccines, local and systemic adverse reactions, any increase in mortality rate and decrease of production were evaluated after the administration to sheep and cattle. The trial was performed according to GCP and GVP.

The age of young cattle belonging to the BLUEVAC BTV8 group ranged between 5.5 and 12.5 months; most of them were either 5.5 or 11-months old. The cattle age distribution for the adults ranged between 20 months and 11 years. The age of young sheep was 3 to 12 months, the age of adult sheep > 12 months. All animals were vaccinated in accordance with the respective vaccination schedule. Thus, cattle were vaccinated with two doses (2 x 4 ml) of BLUEVAC BTV8 at an interval of 21 days. Sheep were vaccinated with one dose (1 x 2 ml). This deviated from the recommended vaccination scheme of two injections. A control group was included receiving a placebo injection. The following table gives an overview on the number of animals involved for the safety evaluation of BLUEVAC BTV8 in the German filed trial.

	CATTLE		SHEEP			
			Farm 1		Farm 2	
	Control	BLUEVAC BTV8	Control	BLUEVAC BTV8	Control	BLUEVAC BTV8
Adults (AA)	201	195	108	78	101	121
Young (YA)	113	103	64	109	51	49
Total	314	298	172	187	152	170

Animals were selected at random from each test group at the start of the study. These animals were examined more closely at fixed points in time. The following parameters were taken into account:

- General findings after individual general examination.
- Rectally measured temperature.
- Local injection site reactions such as pain, swelling, redness, increased temperature, hair and skin changes.

In addition to the standard safety monitoring, milk production yield rate data for cattle were provided and serology results from testing twice for sheep and three times for cattle.

The results of the monitoring confirmed the findings of the laboratory studies with regard to a potential slight increase in body temperature after vaccination and transient swellings at the injection site. The serological data differentiated by young and adult animals indicated an almost 100% sero-conversion in cattle after the two vaccinations. Sheep of the one farm showed also a comparable sero-conversion rate. Animals of the other sheep farm showed lower sero-conversion rates. The milk yield rate for cattle after the 1st vaccination was compared with the yield 52 days before and revealed no reduction in the yield.

Conclusions: The data provided were acceptable in order to support the safety of the product in the field as the vaccine was well tolerated.

User safety

The risk of human exposure is limited to the person injecting the product to the animals (veterinarians, or experienced persons working under the direct supervision of a veterinarian). However the amount and method of administration does not pose any additional risks compared to other injectable products to animals and humans. In the absence of irritating substances such as oil adjuvants, the accidental injection of the vaccine into subcutaneous or muscular tissue does not present any specific lesion risks when accidentally injected. In the absence of any demonstrated risks, according to the provisions in the Guideline on User safety for immunological veterinary medicinal products (EMEA/CVMP/54533/06), section 4.5 of SPC was updated accordingly.

Environmental safety

A Phase I environmental risk assessment was carried out, providing evidence that there would be no potential risk for the global environment. No phase II assessment was deemed necessary. No hazard

should be posed to the environment in light of the nature (inactivated vaccine), and composition of the vaccine under study and the robust manufacturing process. The manufacturing process includes a fully validated inactivation test and control of inactivation and thus there is no risk arising from the presence of live bluetongue virus in the vaccine and its excretion into the environment. The excipients consist of substances that will likewise not be excreted into the environment. The vaccine is contained in high density polyethylene bottles with bromobutyl stoppers. The probability that the accidental opening of a vial may become a risk to the environment is insignificant. In the event that the vaccine reaches the environment, such as in the case of vial breakage or spilling of vaccine, the amounts will be very small and, therefore, it is not expected that such situation will have any measurable effects on the environment. Based on these considerations, the general risk posed to the environment by the use of the vaccine under consideration is negligible.

B. Residue assessment

Study of residues

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

MRL

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

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

Withdrawal period

Cattle: Zero days

Sheep: Zero days

Overall conclusions on safety

In general, sufficient data were provided in order to specifically assess the potential risks arising from the use of BLUEVAC BTV8 in sheep and cattle. Laboratory studies with the vaccine under application were provided that showed that adverse reactions are limited to an average increase in body temperature varying between 0.5 and 1.0 °C in cattle and sheep lasting no longer than 24 to 48 hours. Transient fever was observed in rare cases. Temporary local reactions (swelling) can occasionally occur at the injection site in the form of a painless nodule of 0.5 to 1 cm in sheep and of 0.5 to 3 cm in cattle which disappears within 14 days, at the latest. The administration of an overdose (double dose) does not cause other adverse reactions than observed after the administration of a single dose, except for the size of the local reaction at the injection site (up to 2 cm in sheep; up to 4 cm in cattle).

Reproductive studies were presented which showed that the vaccine can be used during pregnancy in ewes and in cows. Safety throughout pregnancy in cows was extrapolated from a study where a similar vaccine with a different serotype was used. There was no negative impact on the milk yield using the

vaccine in lactating cows. Since no data are available on the safe and efficacious use of the vaccine in breeding males, its use should only be considered after a thorough benefit-risk-assessment.

Currently there are no data related to compatibility and interactions of BLUEVAC BTV8. Therefore, the appropriate warnings are included in the Summary of Product Characteristics (SPC).

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

Appropriate warnings have been placed in the SPC to warn of the potential risks to the target animals. Standard SPC warning sentences have been included in the SPC regarding user safety and environmental safety.

4. Efficacy assessment

BLUEVAC BTV8 is an inactivated vaccine against bluetongue virus serotype 8 indicated for the active immunisation of sheep from 2.5 months of age to prevent viraemia and to reduce clinical signs caused by BTV serotype 8. It has an onset of immunity of 20 days after the second vaccination and duration of immunity of one year. For cattle the vaccine is indicated for the active immunisation of cattle from 2.5 months of age to prevent viraemia caused by BTV serotype 8 with an onset of immunity of 31 days after the second vaccination and duration of immunity of one year.

The recommended vaccination scheme is 2 doses of 2 ml for sheep and 2 doses of 4 ml for cattle at an interval of 21 days. The minimum effective titre is specified as $10^{6.5}$ $TCID_{50}$ per ml prior to inactivation.

A challenge model was established and the suitability of such a model for both sheep and cattle was demonstrated. BTV serotypes 1, 4 and 8 were examined in this model.

Detailed information about origin and passage history of the challenge strains used was provided including information on their relevance. For the monitoring procedures after challenge the parameters assessed and test methods used were similar in all the laboratory efficacy studies (comparison between vaccinated and placebo treated animals).

Laboratory studies:

The trials were performed in accordance with Directive 2001/82/EC as amended. 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) were taken into consideration.

The Onset of Immunity (OOI) challenge studies were carried out in the target species, cattle and sheep, at the minimum age of 2.5 months. The batches (BLUEVAC BTV8) used were representative for production. The virus titres pre-inactivation ranged between $10^{6.0}$ and $10^{6.5}$ $TCID_{50}$ per ml. Data supporting the duration of immunity were provided for CZV's BTV -1, BTV-1+4 vaccines and Bluetongue Vaccine A (serotype 4) as well. These vaccines are of identical qualitative and quantitative composition to the company's BTV8 vaccine except for the serotypes included. New Duration of Immunity (DOI) studies (BLUEVAC BTV8) were provided where BLUEVAC BTV8 batches with a pre-inactivation virus titre of $10^{6.5}$ $TCID_{50}$ per ml were used.

Onset of immunity (protection)

SHEEP

Four laboratory studies in sheep were initially provided to support the claim of prevention of viraemia and reduction of clinical signs. Relevant parts of studies that have been performed for the determination of the most effective dose and in the context of the batch potency test were also taken into consideration to decide on the minimum acceptable period of time between primary vaccination and onset of immunity.

Efficacy (and safety) study on the administration of a single dose and a repeated dose of BLUEVAC BTV8 in sheep (study 1)

BTV-seronegative sheep were vaccinated according to the vaccination schedule. Another group of sheep were left as controls and received a subcutaneous application of placebo.

Vaccine	Vaccination regimen	Dose
BLUEVAC BTV8	2 x 2 ml, 21 days; SC	Lower dose

Only the efficacy related part is presented here.

The challenge took place approximately 30 days after administration of the second dose.

Serology: After challenge, the controls became sero-positive from day 7 post infection.

Viraemia: All the sheep were confirmed RT-PCR negative before challenge. The control animals were positive from the third day of infection and persisting until the end of the observation period on day 27. The vaccinated animals were RT-PCR negative for the whole study period.

Clinical signs: The control animals developed temporarily an increase in body temperature above 41 °C that was significantly higher compared with the temperatures of the vaccinated animals. In addition, they developed clinical signs three days after the infection persisting until day 15 post virus inoculation. The vaccinated group showed no pyrexia at any time. They developed only slight mucous nasal discharge and sporadic coughing.

Conclusions: Protection of sheep of the minimum recommended age with a lower dose of vaccine against viraemia after experimental infection with BTV serotype 8 was demonstrated; however complete prevention of clinical signs was not observed for the sheep. Therefore, the indication was amended to reflect the above and state that the vaccine is capable of reducing clinical signs in sheep rather than preventing them.

Efficacy and safety study on the administration of one single dose BLUEVAC BTV8 to sheep (study 2).

The purpose of this study was to assess the efficacy (and safety) in sheep of minimum age (2.5 months) after subcutaneous administration of one single dose.

BTV-seronegative sheep were vaccinated with one single standard dose. The other group of sheep were left as controls and received a subcutaneous application of placebo.

Vaccine	Vaccination regimen	Dose
BLUEVAC BTV8	1 x 2 ml, SC	Standard dose

The challenge took place 24 days after administration of the single dose.

Serology: After challenge the vaccinated were constantly sero-positive and the controls were sero-positive from day 7 post challenge.

Viraemia: All animals were RT-PCR negative before challenge. The control animals were positive from the third day of infection persisting until the end of the observation period on day 27. The vaccinated animals were RT-PCR negative for the whole study period.

Clinical signs: The control animals developed clinical signs three days after the infection persisting until day 15 post virus inoculation. The vaccinated animals developed only slight mucous nasal discharge and sporadic coughing. The vaccinated group showed no pyrexia at any time whereas the control animals temporarily developed an increase in body temperature above 41 °C that was significantly higher compared with the temperatures of the vaccinates.

Conclusions: The vaccination was done with only one dose. However, the outcome of this study was comparable to the outcome of the previous study in sheep, supporting the efficacy of the vaccine in preventing viremia and reducing clinical signs in sheep.

Final report on dose effect study BLUEVAC BTV8 in sheep (study 3)

Several dose response studies have been carried out to demonstrate the efficacy of the vaccine and to verify the recommended vaccination scheme. Batches were used that contained the intended fixed antigen input titre.

In addition to a control group of sheep, altogether five groups of animals were included. Here, only the group relevant for the onset of immunity to be stated in the SPC and package insert is presented.

BTV free and seronegative sheep of minimum vaccination age were vaccinated twice at an interval of 21 days. The controls received a subcutaneous application of placebo.

Vaccine	Vaccination regimen	Dose
BLUEVAC BTV8	2 x 2 ml, SC	Standard dose

The challenge took place 21 days after administration of the repeated dose.

Serology: The controls were found BTV-seropositive between day 7 and 10 post infection. The anti-VP7 antibody titres of the vaccinated animals increased post infection three weeks later.

Viraemia: All animals were RT-PCR negative before challenge. Animals of the control group were positive from the fourth day of infection persisting until the end of the observation period on day 28. As regards the vaccinated animals, Ct values remained negative throughout the whole observation period.

Clinical signs: The vaccinated sheep showed no pyrexia at any time whereas the control animals developed an increase in body temperature up to and above 41 °C on day 7 post infection. In addition, the control animals developed clinical signs whereas only some of the vaccinated sheep sporadically developed serous nasal and/or ocular discharge. Data were reassessed using the clinical score system.

Conclusions: The outcome of this study was comparable to the outcome of the previous efficacy studies in sheep, supporting an onset of immunity at approximately day 20 after primary vaccination. Prevention of viremia and reduction of clinical signs in sheep was demonstrated.

Final report on dose effect study BLUEVAC BTV8 in sheep (study 4)

Several dose response studies have been carried out to demonstrate the efficacy of the vaccine and to verify the recommended vaccination scheme. Batches were used that contained the intended fixed antigen input titre.

In addition to a control group of sheep, altogether eight groups of animals were included. Here, only the group (V) relevant for the onset of immunity to be stated in the SPC and package insert is presented.

BTV free and seronegative sheep of minimum vaccination age were vaccinated twice at an interval of 21 days. The controls received a subcutaneous application of placebo.

Vaccine	Vaccination regimen	Dose
BLUEVAC BTV8	2 x 2 ml, SC	Standard dose

The challenge took place 20 days after administration of the repeated dose.

Serology: Anti-VP7 antibodies were detectable in the control sheep from day 7 of the infection throughout the whole observation period of 28 days. The vaccinated animals showed a titre increase after the challenge.

Viraemia: All animals were RT-PCR negative before challenge. Animals of the control group were positive from the 2nd and 4th day of infection persisting until the end of the observation period on day 28. As regards the vaccinated animals, Ct values remained negative throughout the whole observation period.

Clinical signs: The vaccinated sheep showed no pyrexia at any time whereas the control animals developed an increase in body temperature up to 40.9 °C on day 7 post infection. In addition, the control animals developed clinical signs whereas only some of the vaccinated sheep sporadically developed very slight serous nasal and/or ocular discharge. Data were assessed using a clinical score system.

Conclusions: The outcome of this study was comparable to the outcome of the previous efficacy studies in sheep, supporting an onset of immunity at day 20 after primary vaccination. Prevention of viremia and reduction of clinical signs in sheep was demonstrated.

CATTLE

Two laboratory studies in cattle were provided to support the claim of prevention of viraemia.

Efficacy (and safety) study of a single dose and a repeated dose of BLUEVAC BTV8 in cattle (study 1)

The purpose of this study was to assess efficacy (and the safety) in cattle of minimum age after subcutaneous administration of a single dose, followed by the administration of a second dose.

BTV seronegative Friesian calves were included in this trial. Two groups were vaccinated subcutaneously as shown in table below. The third group remained as control. Vaccination followed the proposed vaccination schedule. The controls received a subcutaneous application of placebo.

Vaccine	Vaccination regimen	Dose
BLUEVAC BTV8 Group I	2 x 4 ml, 21 days; SC	Lower dose
BLUEVAC BTV8 Group II	2 x 4 ml, 21 days; SC	Standard dose

The challenge took place 36 days after administration of the second dose.

Serology: At the time of challenge, 36 days after the second vaccination and 57 days after the first vaccination respectively, positive virus neutralisation titres were observed in each vaccination group. The controls remained negative.

The qualitative anti-VP7 antibody ELISA was performed covering the entire blood sampling period from day 0 post challenge until 27 days post challenge. Controls were found to be antibody positive from day 17 after the infection.

Viraemia: All animals were RT-PCR negative before challenge. Animals of the control group were positive from the third day after infection persisting until the end of the observation period on day 27. As regards the vaccinates, Ct values considered to be clearly negative for infection were observed except for one animal that showed an inconclusive result but only for one day. In agreement with the definitions, it was not considered to be indicative for viraemia.

Clinical signs: No clinical signs were observed either in the vaccinated or in the controls. This is deemed to be in line with previous findings regarding BTV challenges in cattle.

Conclusions: The vaccine was considered to be protective against the development of viraemia. As no difference was observed between vaccinates and controls no claim could be supported for reduction of clinical signs in cattle.

Efficacy (and safety) study of a single dose (2 ml) and a repeated dose (2 ml) of BLUEVAC BTV8 in cattle (study 2)

The purpose of this study was to assess the efficacy (and the safety) in cattle of minimum age after subcutaneous administration of a single dose, followed by the administration of a second dose. It deviated from the regular vaccination protocol; one dose consisted of 2 ml instead of 4 ml.

BTV seronegative Friesian calves aged 2 months were vaccinated subcutaneously as shown in table below. Other group remained as control. The controls received a subcutaneous application of placebo.

Vaccine	Vaccination regimen	Dose
BLUEVAC BTV8	2 x 2 ml, 21 days; SC	Half of lower dose

The challenge took place 31 days after administration of the second dose.

Serology: At the time of challenge, 31 days after the second vaccination, a positive virus neutralisation response was observed in the vaccination group. The controls remained negative. However, after infection controls showed a positive response from day 11 in the qualitative anti-VP7 antibody ELISA.

Viraemia: All animals were RT-PCR negative before challenge. Animals of the control group were positive from the third day after infection persisting until the end of the observation period on day 27. As regards the vaccinated, cycle threshold (Ct) values considered to be clearly negative for infection, were observed in 75% of animals. One animal showed a low level of Ct values in two consecutive tests. Since the values are close to the limit of detection, they were considered to be indicative for transient viraemia

Clinical signs: No relevant clinical signs were observed either in the vaccinated or in the controls.

Conclusions: This study deviated from the proposed vaccination scheme and results showed a transient viraemia in vaccinates. On the basis of the above this study could not be supportive of the vaccine's efficacy in cattle.

Overall conclusions on the onset of immunity studies: The onset of immunity was considered to begin with the challenge date which for sheep was on day 20 after the second dose, and for cattle on day 31 and 36 respectively after the second dose.

Duration of immunity (DoI)

Initially, duration of immunity studies were provided that were using CZV's BTV vaccines with other serotypes for sheep (DoI of 8 months) and for cattle (DoI of 7 months). Both studies were considered of a supportive nature only.

The most relevant DoI studies using BLUEVAC BTV8 batches are presented below and they were carried out in both sheep and cattle.

SHEEP

Duration of immunity study in sheep of minimum age

Sheep of minimum age were vaccinated twice at an interval of 21 days. The BLUEVAC BTV8 batch used contained the currently fixed pre-inactivation titre of $10^{6.5}$ $CCID_{50}$ per ml.

Challenges were performed approximately 126 and 284 days after administration of the second dose. The efficacy was assessed by serology, protection against viraemia and clinical signs.

Results: Anti-VP7 antibodies were detectable at the time of challenge after the second vaccination. All vaccinated animals remained BTV negative throughout the course of the observation period as indicated by the Ct values obtained. In relation to the clinical outcome, though some of the vaccinated sheep developed sporadically a slight transient increase in body temperature and slight serous nasal/ocular discharge, there was a significant difference when compared with the controls which presented more severe signs.

Conclusions: For sheep prevention of viraemia is considered to be proven as well as at least the reduction of clinical signs for up to 10 months after the second vaccination.

SHEEP and CATTLE

Duration of immunity study in cattle and sheep

The study provided was a continuation of the study initiated by the Animal Health German Authorities (LALLV/MV) in collaboration with the Friedrich-Loeffler-Institute (Federal Research Institute for Animal Health) to assess the safety and efficacy of several BTV serotype 8 vaccines in spring 2008. Three different vaccines were assessed in sheep and cattle one year after the vaccine administration.

The vaccines were tested in a Holstein-Friesian dairy cattle farm and in two sheep farms (German black-headed Mutton sheep). Cattle were vaccinated with two doses (4 ml each) at an interval of 21 days, sheep with one dose (2 ml) one year before the starting of this DoI study. A BLUEVAC BTV8 batch with a virus titre lower than the standard dose per ml was used. Prior to challenge the animals were tested for BTV freedom by real time RT-PCR to verify that no BTV had occurred since 2008. Furthermore, serological tests were conducted using an ELISA test kit and a SN test.

An European BTV8 field isolate originating infectious bovine blood was used in the challenge.

Results: The control animals developed antibody titres 14 days after challenge, while vaccinated animals displayed a clear booster reaction seven days post challenge. Control sheep and cattle developed increased body temperatures 5 days post challenge lasting for 5 – 10 days. The vaccinated animals showed no febrile reaction. The BTV genome was detected in all but one control animal, whereas no virus genome was detected in vaccinated cattle and sheep except for one cow which scored positive but at a lower level than the controls. Virus was isolated from all RT-PCR positive animals.

Conclusions: Protection against viraemia was induced in sheep and cattle one year after the administration of the second dose of BLUEVAC BTV8.

The influence of maternal antibody on the efficacy of the vaccine.

The efficacy of the vaccine in the presence of maternally derived antibodies was not investigated. A warning has been included in the relevant section of SPC.

Field trials

A field trial was conducted in Germany with the collaboration of the Federal Research Institute for Animal Health (Friedrich-Loeffler Institute). The safety and efficacy profile of several BTV vaccines were assessed prior to the extensive use in the German field in 2008. The trial was performed according to Good Clinical Practice (GCP) and Good Veterinary Practice (GVP).

The vaccines were tested in a cattle farm of 298 cattle and in two sheep farms of 357 sheep. Clinically healthy BTV antibody negative animals from an age of at least 3 months onwards were included in the study. The animals were allocated in three groups according to the manufacturers. Only clinically healthy animals from an age of at least 3 months onwards were included in the study. The age of young cattle belonging to the CZV group ranged between 5.5 and 12.5 months; most of them were either 5.5 or 11-months old. The cattle age distribution for the adults ranged between 20 months and 11 years. The age of young sheep was 3 to 12 months; the age of adult sheep was more than 12 months. All animals were vaccinated in accordance with the respective vaccination schedule. Thus, cattle were vaccinated with two doses (2x4 ml) of BLUEVAC BTV8 at an interval of 21 days. Sheep were vaccinated with one dose (1x2 ml). This deviates from the currently requested two injections. A control group was included receiving a placebo injection.

Monitoring after vaccination: Forty animals were selected at random from each test group at the start of the study. These animals were examined more closely at fixed points in time. The following parameters were taken into account:

- General findings after individual general examination.
- Rectally measured temperature.
- Local injection site reactions.
- Milk production yield rate data for cattle.
- Serology performed by cELISA, twice for sheep and three times for cattle.

A total of 23 sheep and 24 cattle were challenged (at least 28 days after the last vaccination). Animals of each species were distributed in three groups (according to the manufacturers) of 6 sheep or cattle respectively. Unvaccinated challenge controls were included. All these animals were moved to the Level 3 biological containment facilities at Friedrich-Loeffler Institute (Riems, Germany). All animals were challenged with 4 ml of blood containing, in high quantity, the current German BTV8 virus.

Results:

Serology: Serological results prior to challenge revealed in all vaccinated animals (sheep and cattle) clear antibody titres against BTV determined by a competitive ELISA except for one sheep (no 16). This sheep had been vaccinated with BLUEVAC BTV8 and did not develop an antibody titre. The administration of the second dose in cattle induced a clear increase of the antibody titres.

Viraemia: The majority of control sheep and the unvaccinated cattle were constantly BTV-positive post infection. No viraemia was detected in any of the vaccinated animals except in one sheep that was also anti VP7 antibody negative at the time of challenge; this sheep clearly expressed positive Ct values.

Clinical signs: Some sheep of the control group showed minor clinical signs of the disease and high temperatures. There were no signs of febrile response in any of the vaccinated animals.

Conclusions: None of the vaccinated cattle presented the BTV8 genome in the blood which showed a good intake of the vaccine. However the vaccination scheme used for the sheep (a single dose of 2 ml) was not in line with the recommended scheme as proposed in the SPC and therefore the study is of supportive nature only for sheep.

Overall conclusion on efficacy

Sheep: The prevention of viraemia and reduction of clinical signs in sheep from 2.5 months after the administration of a primary course (2 x 2 ml) were considered to be demonstrated. The onset of immunity can be set at 20 days after the second vaccination. The duration of immunity is 12 months based on the results of the DoI study that was carried out at Friedrich-Loeffler-Institute, Germany.

Cattle: Vaccination of cattle from 2.5 months with a primary course (2 x 4 ml) induced prevention of viraemia after challenge. The onset of immunity can be set at 31 days after the second vaccination. The duration of immunity can be assumed to be approximately 12 months taking into account the results of the DoI study carried out at Friedrich-Loeffler-Institute, Germany.

Since there was no information available on the use of the vaccine in seropositive sheep or cattle, including those with maternally derived antibodies, its use should be undertaken with care. The same care should be undertaken if the vaccine is used in other domestic or wild ruminant species that are considered at risk of infection. The efficacy of the proposed vaccination scheme in breeding males and the impact of the acquired maternal immunity on the efficacy when vaccinating young animals, were not investigated. Therefore specific warnings in the relevant sections of the SPC were included. The efficacy in pregnant animals was also not investigated, but the safety was assessed and demonstrated. All these circumstances have been reflected in the SPC. Neither DIVA strategy was implemented, nor data were provided in relation to the development of any strategy allowing the differentiation between infected and vaccinated animals.

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

5. Benefit risk assessment

Introduction

BLUEVAC BTV8 is an inactivated vaccine prepared from a bluetongue serotype 8 strain isolated in 2006 from an outbreak in Belgium. 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. In view of the epidemiological situation and the potential for epizootic spread if urgent measures, including vaccination, are not taken to control the disease at EU level, this application has been considered for an authorisation under exceptional circumstances.

Benefit assessment

Direct therapeutic benefits

BLUEVAC BTV8 is a vaccine containing inactivated BTV8 combined with an adjuvant intended to induce an immune response in sheep and cattle from 2.5 months of age in order to prevent viraemia and to reduce clinical signs caused by bluetongue virus serotype 8 in sheep and to prevent viraemia caused by bluetongue virus serotype 8 in cattle. The onset of immunity is 20 days after the completion of the basic vaccination course for sheep and 31 days for cattle and duration of immunity of 12 months for both species has been adequately supported.

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 in both sheep and cattle and to decrease the impact of clinical signs for sheep.

Additional benefits

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

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

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

In cattle, the safety during pregnancy was extrapolated by a study that was conducted in pregnant cows with a similar vaccine to BLUEVAC BTV8 which contained a different serotype (BTV1). Vaccination was also shown to be safe for use during lactation in cattle, as the milk yield was not negatively impacted. In addition, no negative impact is to be expected in the milk production of lactating ewes.

Risk assessment

Main potential risks:

- For sheep and cattle there is a risk of a slight rise in temperature (between 0.5 and 1.0 °C) which lasts no longer than 24-48 hours. Temporary swellings at the injection site may occur following vaccination. These swellings may last for over 14 days in sheep and cattle. The SPC wording is adequate in relation to this matter.
- For the user there is a low risk of self injection. However in the absence of irritating substances such as oil adjuvants, the accidental injection of the vaccine into subcutaneous or muscular tissue does not present any specific risks when accidentally injected.
- 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 over failure to observe an MRL. The product contains components found in other marketed products and therefore the risk is no greater than already exists.

Specific potential risks, according to product type and application

- Limited data are available on the stability of product during storage. Under exceptional circumstances a preliminary shelf life of 12 months to be granted for this product is acceptable. Nevertheless, there might be a minimum risk that the product is not stable during this period. Due to the need to provide authorised vaccines against Bluetongue serotype 8 as soon as possible and the considerable time required in order to complete all stability studies in line with normal requirements the CVMP can exceptionally accept the current limited data with a view for the applicant to provide the remaining information as soon as available.
- There is no information available concerning the use of the vaccine in sero-positive sheep and cattle (antibodies derived maternally or after infection).

Risk management or mitigation measures

Appropriate warnings have been placed in the SPC to warn of the potential risks to the target animals. Standard SPC warning sentences have been included in the SPC regarding user safety and environmental safety.

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

Evaluation of the benefit risk balance

BLUEVAC BTV8 has been shown to have a positive benefit risk balance for use in sheep and cattle. This vaccine has been shown to be efficacious for the indication regarding prevention of viraemia and reduction of clinical signs in sheep and for the prevention of viraemia in cattle.

The formulation and manufacture of BLUEVAC BTV8 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.

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

Conclusion on benefit risk balance

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

- Bluetongue disease is spread by insect vectors and therefore presents particular challenges in terms of control due to an inability to prevent transmission from infected animals other than through insect control combined with reducing or preventing viraemia (virus in the blood) in susceptible animals by means of vaccination.
- Bluetongue disease is epizootic in nature and has the potential to result in high morbidity and mortality in susceptible populations, particularly of sheep.
- Over the last ten years the bluetongue epidemiological situation in Europe has changed considerably, with the incursion of new serotypes that have been never reported before and

with outbreaks in areas which until now were not considered at risk of bluetongue, as is the case for BTV serotype 8. Also with the outbreaks of BTV-1 declared in Spain, Portugal and France and the possibility of spread of this serotype to other regions and countries in Europe is notice and the possible co-infection with both serotypes has also occurred, with not well studied consequences of the epidemiology and pathology of the disease.

- There are still a small number of vaccines against bluetongue in Europe.
- That consequently any delay should be avoided where possible in making available safe and effective vaccines that have been demonstrated to be in compliance with the CVMP guideline on Minimum Data Requirements for an Authorisation under Exceptional Circumstances for Vaccines for Emergency Use against Bluetongue (EMEA/CVMP/IWP/220193/2008).

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

- The applicant cannot reasonably be expected to provide the results from certain trials on the target species due to the difficulties in conducting large scale trials for a disease that is under community control and the need for any experimental studies to be conducted within high containment facilities.
- Considerable time is required in order to complete all stability studies in line with normal requirements and therefore the CVMP could exceptionally accept the current limited data with a view for the applicant to provide the remaining information as soon as available.

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 as amended.