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Veterinary Medicines Division

## Committee for Medicinal Products for Veterinary Use (CVMP)

# CVMP assessment report for Syvazul BTV (EMEA/V/C/004611/0000)

Common name: Bluetongue virus vaccine (inactivated) (multi-strain: 1-2 strains out of a set of 3)

**Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted**

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# Introduction

The applicant LABORATORIOS SYVA, S.A.U. submitted on 5 April 2017 an application for a marketing authorisation for a multi-strain dossier to the European Medicines Agency (the Agency) for Syvazul BTV through the centralised procedure under Article 3(2)b of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 8 September 2016 as the applicant showed that the product would be in the interest of animal health at Community level, as increasing availability of bluetongue inactivated vaccines with a multi-strain dossier would represent a helpful tool in the control of the dissemination of the relevant serotypes through the EU.

Syvazul BTV is a bluetongue virus (BTV) vaccine (inactivated) (multi-strain: 1-2 strains out of a set of 3) for active immunisation of sheep and cattle against BTV serotypes 1, 4 and 8. Each dose is formulated to contain the relevant serotype(s) at a Relative Potency (RP) ≥ 1. The product is a suspension and is intended for administration by intramuscular injection in cattle and by the subcutaneous route in sheep.

The vaccine is intended for the following indications:

*Sheep:*

For active immunisation of sheep to prevent viraemia* and reduce clinical signs and lesions caused by bluetongue virus serotypes 1 and/or 8 and/or to reduce viraemia* and clinical signs and lesions caused by bluetongue virus serotype 4 (combination of maximum 2 serotypes).

Onset of immunity: 39 days after completion of the primary vaccination scheme.

Duration of immunity: one year after completion of the primary vaccination scheme.

*Cattle:*

For active immunisation of cattle to prevent viraemia* caused by bluetongue virus serotypes 1 and/or 8 and/or to reduce viraemia* caused by bluetongue virus serotype 4 (combination of maximum 2 serotypes).

Onset of immunity: 21 days after completion of the primary vaccination scheme.

Duration of immunity: one year after completion of the primary vaccination scheme.

*Below the level of detection by the validated RT-PCR method at 1.32 $\log_{10}$ $TCID_{50}$/ml

Syvazul BTV is presented in polypropylene vials containing 80 or 200 ml. Package sizes: Cardboard box with 1 vial containing either 40 sheep doses or 20 cattle doses (80 ml). Cardboard box with 1 vial containing either 100 sheep doses or 50 cattle doses (200 ml).

The applicant is registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC.

The rapporteur appointed was Noemi Garcia del Blanco and the co-rapporteur was Gábor Kulcsár.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 8 November 2018, the CVMP adopted an opinion and CVMP assessment report.

On 9 January 2019, the European Commission adopted a Commission Decision granting the marketing

authorisation for Syvazul BTV.

### *Marketing authorisation under exceptional circumstances*

Not applicable.

### *Scientific advice*

Scientific advice was given concerning the need to perform efficacy field studies for Syvazul BTV multi-strain vaccine (which at the time could contain two different strains of the virus, BTV-1 and BTV-8; BTV-4 was incorporated to the multi-strain vaccine in a later stage of the development) taking into account the already widespread use of their serotype 1 and serotype 8 vaccines and the epidemiological situation where exposure to natural infection during a field trial would be unlikely (EMA/CVMP/SAWP/434002/2013). The applicant had stated an intention to carry out field safety studies in various animal categories of the two target species. The CVMP advised to monitor the production of neutralising antibody titres in vaccinated animals as an indicator of efficacy of the vaccine during the planned safety field trials so that the antibody titres that develop in the field trials could then be compared to the titres observed in the corresponding laboratory trials.

### *MUMS/limited market status*

Not applicable.

### *Multi-strain dossier*

The application has been submitted in accordance with Annex I to Directive 2001/82/EC as amended and for which CVMP has published a Guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD); (EMA/CVMP/IWP/105506/2007). Three strains of BTV are included in the dossier, namely BTV serotypes 1, 4 and 8. The vaccine may contain up to 2 serotypes of inactivated viral antigens adjuvanted with aluminium hydroxide and saponin, chosen from the 3 serotypes included in the dossier depending on epidemiological need.

## Part 1 - Administrative particulars

### *Detailed description of the pharmacovigilance system*

A detailed description of the pharmacovigilance system (dated March 2016, Annex 5.20), which fulfils the requirements of Directive 2001/82/EC, was provided. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

### *Manufacturing authorisations and inspection status*

Manufacture of the active substance and finished product, including all packaging, and batch release takes place at LABORATORIOS SYVA S.A.U., Avda. Portugal S/N, Parcelas M15-M16, León (Spain). The site has a manufacturing authorisation issued on 3 April 2017 by the Spanish Agency of Medicines and Medical Devices. Good Manufacturing Practice (GMP) certification has been provided to confirm that the site is authorised for the manufacture of such veterinary dosage forms, with the date of the last

inspection being May 2017.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by the QA Team LABORATORIOS SYVA S.A.U., which has taken into consideration the GMP certificate available for the active substance site issued by the Spanish Agency of Medicines and Medical Devices.

### *Overall conclusions on administrative particulars*

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

Following the provision of a valid GMP certification of the manufacturing site, it can be concluded that the GMP status of the active substance and of the finished product manufacturing site have been satisfactorily established, and are in line with legal requirements.

# Part 2 – Quality

## *Chemical, pharmaceutical and biological/microbiological information (quality)*

### *Qualitative and quantitative particulars of the constituents*

### *Qualitative and quantitative particulars*

The vaccine may contain either one or two of the following inactivated bluetongue virus strain antigens at concentrations equivalent to RP ≥ 1 per dose (RP=relative potency compared to a reference vaccine that was shown efficacious in sheep and confirmed relevant for cattle):

- BTV, serotype 1, strain ALG2006/01 E1
- BTV, serotype 8, strain BEL2006/01
- BTV, serotype 4, strain BTV-4/SPA-1/2004

The vaccine contains aluminium hydroxide and saponin as adjuvants, thiomersal as preservative, as well as other excipients including silicone antifoam and phosphate buffered saline (PBS) (potassium chloride, sodium chloride, disodium phosphate hydrogen - anhydrous, potassium phosphate dihydrogen and water for injections).

The quantities of active ingredients per ml in terms of ELISA units and corresponding volumes, which apply to all the possible combinations (maximum of two of three serotypes) included in Syvazul BTV, were provided. This fits within the context and purpose of a multi-strain dossier.

### *Containers and closures*

The product is aseptically filled into pre-sterilised 100 or 250 ml polypropylene vials closed with bromobutyl rubber stoppers and subsequently sealed with an aluminium cap using fill volumes of 80 ml and 200 ml, respectively. Documentation in support of the specifications and sterilisation of the vials and stoppers were provided. These documents confirmed that the vials and stoppers are in compliance with pharmacopoeial standards.

The pack /container sizes are consistent with the vaccination schedule and intended use.

## *Product development*

Syvazul BTV has been developed in accordance with the multi-strain dossier concept introduced in the revised Annex I to Directive 2001/82/EC. The three strains of BTV virus that may be incorporated into the finished product, depending on epidemiological need, have been selected by the applicant based on the epidemiological situation of bluetongue disease in the EU.

Vaccine strain ALG2006/01 E1 (BTV-1) was isolated from the spleen of an infected sheep which originated from Algeria. Almost total genetic homology has been demonstrated between this strain and the field strain responsible for outbreaks in the south of Spain from 2007. Vaccine strain BEL2006/01 (BTV-8) was isolated from blood samples from sheep during a disease outbreak of bluetongue disease in the north and centre of Europe in 2006. Vaccine strain BTV-4/SPA-1/2004 (BTV-4) was isolated from sheep during a disease outbreak of bluetongue disease in the Spanish region of Andalucía in 2004. The information provided by the applicant in support of the relevance of the BTV vaccine strains in particular to the current epidemiological situation of bluetongue disease in the EU is considered sufficient. The inclusion of the strains is in line with the guideline EMA/CVMP/IWP/105506/2007. The applicant initially authorised BTV-1, BTV-8 and BTV1+8 under temporary authorisation for emergency use in Spain, before following the multi-strain dossier approach with the inclusion of BTV-4.

Formulation of these vaccines produced for emergency use was initially based on antigen load before inactivation. Specific ELISA tests were developed to allow differential quantification of the inactivated BTV-1 and BTV-8 antigen and the results of these tests were found to be highly correlated with those obtained by titration prior to inactivation. BTV-4 was included at a later stage, including an ELISA test to quantify the antigen, based on the experience with BTV-1 and BTV-8. The fixed formulation is based on ELISA units post inactivation instead of $TCID_{50}$/ml.

Viral antigens are inactivated by treatment with binary ethylenimine (BEI) and residual inactivant is neutralised with sodium thiosulphate. Three studies have been presented as part of the validation of the inactivation kinetics for the different serotypes, with inactivation demonstrated within a period of time less than 67% of the inactivation process, which complies with the European Pharmacopoeia (Ph. Eur.) requirements.

The batches used in the studies were produced according to Part 2B of the dossier and batch records have been provided. A test for complete inactivation is performed by three passages in baby hamster kidney cells (BHK-21). Three studies have been presented as part of the validation of the test for the different serotypes. In principle, the method for each of the serotypes is considered adequately validated and the sensitivity for the different serotypes is satisfactory. When the maximum pre-inactivated titre proposed is greater than that established in the inactivation kinetics studies, a pre-dilution step with Phosphate Buffered Saline plus Tween (PBST) is employed to ensure pre-inactivation titre is within the validated range.

The *in vitro* potency test, which is the same ELISA test used to quantify the inactivated antigen, is determined by comparison to reference monovalent vaccines known to be efficacious in sheep and the relevance of these references for cattle was confirmed. The vaccine is adjuvanted with aluminium hydroxide and saponin, selected for its ability to stimulate immunity whilst keeping an acceptable safety profile in the target species.

All other excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC.

The target quantities of each of the respective antigens per dose have been determined on the basis of the results of a number of safety and efficacy studies carried out in the target species.

The vaccine contains thiomersal, intended to minimise the risk of contamination and degradation of the vaccine during the use of the multi-dose containers (vials).

## *Description of the manufacturing method*

The vaccine is manufactured by a relatively standard procedure, which is identical for all the three different BTV strains. Vaccine production is under GMP and sterility is assured with in-process and finished product testing. The BTV strains are cultured in baby hamster kidney cells (BHK-21) in culture flasks.

Gentamicin was used in the production of the active ingredient as a component of cell culture medium during cell amplification and during viral antigen production and was present in the final product at a concentration higher than trace amounts, and that exceeded the established MRLs in both target species. To address this concern, it was proposed to remove gentamicin from the culture medium used during viral antigen production and therefore it will not be present in the antigen or in the finished product. A risk assessment was provided to demonstrate that the proposed removal of gentamicin is not expected to have any effect on the quality of the vaccine and the proposal was considered acceptable with recommendations to produce and test three batches of antigen (one of each of the serotypes), to verify the suitability of the sterility test for product without gentamicin and to verify that the removal of gentamicin has no impact on in-use stability by repeating the test for efficacy of antimicrobial preservative after manufacture of a batch of vaccine without gentamicin.

Viral antigens are inactivated by treatment with binary ethylenimine (BEI). After inactivation, the residual inactivant is neutralised with sodium thiosulphate. Validation of the inactivation process was demonstrated with three or four production scale batches of each of the antigens. Confirmation that the batches used in the validation studies were produced according to Part 2B of the dossier has been provided. Antigen bulks can be stored at 5±3 °C for a maximum of 12 months. Data to support the storage of the bulk antigen for this period of time have been presented and considered adequate.

To formulate the finished product, the selected combinations of one to two antigens are mixed with the adjuvants and excipients. A fixed antigen input for each of the BTV antigens has been established for formulation based on ELISA units which have been correlated with pre-inactivation titres and efficacy. A blending table has been provided based on composition per ml, which can be used to calculate any batch of monovalent or bivalent vaccine. A real working example of blending, including details of calculations from pre-inactivation titres, post-inactivation ELISA units and calculations/dilutions needed in order to reach the fixed antigen input required for blending of a BTV mono-strain vaccine, has been provided. In addition, information on how this same process varies when blending a multi-strain vaccine has also been provided.

The method of manufacture was well described.

Validation of the production process as a whole was demonstrated with the provision of results from three consecutive production scale batches of each of the mono-strain vaccines BTV-1, BTV-4 and BTV-8, produced using the method described.

## *Production and control of starting materials*

## *Starting materials listed in pharmacopoeias*

Certificates of analysis have been provided for all starting materials listed in pharmacopoeias and all conform to the specifications in the specific monographs.

## *Specific materials not listed in a pharmacopoeia*

## *Starting materials of biological origin*

Detailed specifications have been provided for all starting materials used to manufacture the vaccine. The only starting materials of biological origin used in the production of the vaccine are virus and cell culture seeds, saponin, trypsin-EDTA solution and tryptose phosphate broth. The BHK-21 cell line has been adequately tested in compliance with Ph. Eur. 5.2.4, including specific tests to demonstrate freedom from extraneous agents in accordance with the recommendations in the monograph and guideline 7BIm10a 'Table of extraneous agents to be tested for in relation to the general and species-specific guidelines on production and control of mammalian veterinary vaccines'. The various BTV master seeds have also been adequately tested according to the relevant requirements (Ph. Eur. 0062, guideline 7BIm10a and Annex 2 of the "Guideline on requirements for the production and control of immunological veterinary medicinal products" (EMA/CVMP/IWP/206555/2010-Rev.1). Where testing was not performed, a risk assessment was provided, which was considered acceptable. However, new tests for three diseases (Schmallenberg virus, endogenous retrovirus and bovine polyoma virus) are under development and the applicant has volunteered to continue the development of these tests as a post-authorisation recommendation. Viral cell seeds are stored at -80 °C and BHK-21 cell seeds are stored in liquid nitrogen.

The adjuvant saponin is not listed in a pharmacopoeia but its quality standard is satisfactory. Certificates of analysis of Trypsin-EDTA solution (used during production for cell detachment) and tryptose phosphate broth (used as a supplement in culture media) have been provided and their quality standards are considered satisfactory.

All of the starting materials of animal origin have been assessed and considered to be in compliance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathies (TSE) agents via human and veterinary medicinal products (EMA/410/01 rev.3). The overall TSE risk associated with the inactivated vaccine is considered negligible.

## *Starting materials of non-biological origin*

Certificates of analysis have been provided for bromoethylamine hydrobromide (used for inactivation), Hepes (component of culture medium), Minimum Essential Medium (MEM) (component of culture medium), silicon antifoaming agent and β-naphthol Violet (pH indicator used in the preparation of the BEI solution) and all conform to in-house specifications.

## *In-house preparation of media and solutions consisting of several components*

The following media and solutions are prepared in-house for use during production of the vaccine: 0.1 M BEI solution, lyophilisation excipient for viral seed, MEM BHK-21 (cell line culture medium) and PBS. Information regarding the qualitative and quantitative composition of these media, their

treatment processes and their storage conditions is provided in the dossier. All components are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

## *Control tests during the manufacturing process*

Control tests carried out during antigen production include: tests on the cell substrates - appearance, passage number and count for new Working Cell Seeds (WCS); tests on the viral antigen - bacterial and fungal sterility, virus titration, inactivation test, quantification of the inactivated antigen (ELISA), residual thiosulphate; tests on the bulk vaccine - pH and sterilty; and tests on the final production of the vaccine (filling and packaging).

Validation of in-process tests is considered satisfactory, following receipt of batch protocols and further clarity on some points. Validation has been provided for the in-process sterility test which is considered acceptable and confirms Ph. Eur. compliance. The applicant has indicated, following the validation of the test used for quantification of the antigen, that the range of specifications for this test is greater than that validated in the test, however, a pre-dilution step with PBST can be done to ensure that the pre-inactivation titre is within the validated range.

## *Control tests on the finished product*

The description of the following methods used for the control of the finished product was provided: appearance, volume, secondary packaging, identification, *in vitro* potency, quantification of saponin and quantification of the adjuvant aluminium hydroxide, thiomersal, sterility and pH.

The specifications proposed for the control tests are considered acceptable.

A specific *in vitro* test has been developed for identification and potency of each of the mono-strain vaccines as recommended in the CVMP Guideline EMA/CVMP/IWP/105506/2007. These separate ELISA tests, specific for each antigen, are also used to quantify the antigen post-inactivation. The *in vitro* method is a double antibody sandwich ELISA-based test, using different pairs of monoclonal antibodies that specifically recognise the VP2 protein of each virus strain. Optical density values obtained (read at 450 nm) are compared with internal reference vaccines specific for each serotype. The reference batches used for BTV-1 and BTV-8 did not appear to have been used in a cattle efficacy study, however, the applicant has supported the relevance of these reference batches for cattle with data from other relevant batches that have been used in cattle efficacy studies.

The ELISA method has been validated for each of the antigens and specificity of the monoclonal antibodies has been confirmed in these studies, with no cross reactions found between strains. Validation of the method for the mono-strain vaccines has also been confirmed following extraction of the vaccine from the aluminium hydroxide, and is acceptable. Validation included a multi-strain vaccine and confirmed the lack of interference with the matrix material included in the test of potency, and is acceptable. Validation of the extraction process for the antigen is confirmed as part of the validation of the potency test.

Information has been provided on the production of the reference batches to confirm that the method of production used is the same as Part 2B. In addition, information has been provided on the monitoring and replacement of the reference batches, as well as how a new reference batch is validated against the old batch.

## *Batch-to-batch consistency*

The consistency of production of the finished product has been supported by data submitted from 3 different batches of mono-strain BTV-1, 3 batches of mono-strain BTV-4 and 3 batches of mono-strain BTV-8. Both vial presentations are represented in the consistency studies, which included 3 different batches with a fill volume of 80 ml and 6 different batches with a fill volume of 200 ml.

## *Stability*

Stability data have been presented for the production seeds and are supportive of the proposed storage period of 4 weeks at 5±3 °C.

Stability data have been presented for the inactivated viral suspensions of BTV-1, BTV-4 and BTV-8. Real-time stability studies were performed using three batches of each of the inactivated antigens. The batches were produced as per Part 2B of the dossier and tested during storage by assessing appearance, sterility and antigen quantification by ELISA. Determination of the antigen content was carried out for each antigen by using the same ELISA tests described to measure the potency of the vaccines.

The data presented are supportive of the proposed storage period of 12 months at 5±3 °C. In order to provide further assurance of stability of antigen, the applicant is recommended to include one batch of final product made with aged antigen into the GMP stability programme.

The stability of the finished product has been demonstrated using a total of nine batches of vaccines, three formulated with each of the mono-strain vaccines, which is in line with the CVMP Guideline EMA/CVMP/IWP/105506/2007. The data presented are supportive of the proposed storage period of 24 months at 5±3 °C. Consequently, the shelf life of 24 months at 5±3 °C for all the possible strain combinations in Syvazul BTV is accepted. The efficacy of the antimicrobial preservative was satisfactorily demonstrated.

## *Overall conclusions on quality*

The qualitative and quantitative particulars of the vaccine suspension and the containers are described adequately. The vaccine has been developed in accordance with the multi-strain dossier concept, introduced in the revised Annex I to Directive 2001/82/EC.

The method of manufacture is satisfactorily described. Validation of the production process was demonstrated with provision of results from consecutive production-scale batches of each of the mono-strain vaccines produced. The production system is a BHK-21 cell culture.

Gentamicin was removed from the culture medium used during final viral antigen production and therefore it will not be present in the final antigen or in the finished product. The removal of gentamicin is not expected to have any effect on the quality of the vaccine. A number of post-authorisation recommendations are given to the applicant to further verification.

Detailed specifications have been provided for all starting materials used to manufacture the vaccine. All starting materials comply with the provisions of Ph. Eur. and the TSE risk is considered negligible.

The in-process and finished product tests for the vaccine are described satisfactorily. The consistency of production of the finished product has been supported by data submitted from 3 different batches.

The results of the analysis of 3 batches of mono-strain BTV-1, 3 batches of mono-strain BTV-4 and 3 batches of mono-strain BTV-8 indicate satisfactory consistency between batches.

Stability data have been presented for the production seeds, for the inactivated viral suspensions of

BTV strains and for the finished product, including in-use shelf life. The provided stability data are satisfactory, however, in order to provide further assurance of stability of antigen, the applicant is recommended to include one batch of final product made with aged antigen into the GMP stability programme.

The proposed shelf life of 24 months for the finished product for all the possible strain combinations with an in-use shelf life of 10 hours is considered demonstrated.

In conclusion, the data provided are supportive of the quality of the Syvazul BTV vaccine.

**Post-authorisation recommendations:**

To verify that removal of gentamicin has no impact on quality and consistency of production, the applicant is recommended to produce and test 3 batches of antigen (one of each of the serotypes) according to the proposed process. The competent authorities should be notified if values outside specifications are seen.

To verify the suitability of the sterility test for product without gentamicin, the applicant is recommended to conduct the Ph. Eur. 2.6.1 suitability test in parallel to the sterility control, performed on the first three batches of antigen and vaccine manufactured, as proposed, without gentamicin.

To verify that removal of gentamicin has no impact on in-use stability, the applicant is recommended to repeat the test for efficacy of antimicrobial preservative after manufacture of a batch of vaccine without gentamicin.

In order to provide further assurance of stability of the antigen, the applicant is recommended to include one batch of final product made with aged antigen into the GMP stability programme.

To continue developing specific tests for Schmallenberg virus, endogenous retrovirus and bovine polyoma virus as volunteered by the applicant, and to provide this information when completed.

# Part 3 – Safety

## *Introduction and general requirements*

Syvazul BTV is a multi-strain dossier for an inactivated and adjuvanted vaccine against bluetongue virus (BTV), containing a maximum of two of three BTV serotypes (BTV-1, BTV-4 and BTV-8) intended for use in sheep and cattle. The number and type of strains included in the final product will be adapted to the current epidemiological situation at the time of the formulation of the final product. The strain combinations that the applicant intends to market at present are: monovalent BTV-1, monovalent BTV-4, monovalent BTV-8, bivalent BTV-1+4, bivalent BTV-1+8 and bivalent BTV-4+8. A fixed target antigen amount of each of the corresponding BTV strain is contained in the vaccine, based on ELISA units, which have been correlated with pre-inactivation titres, so the relative potency for each strain in the finished product is greater than or equal to one (RP ≥ 1). The relative potency is estimated by ELISA in relation to a reference vaccine whose efficacy has been demonstrated by challenge in the target species. The vaccine is adjuvanted with aluminium hydroxide and saponin.

In sheep, the vaccine is intended for active immunisation of animals from 3 months of age and is administered subcutaneously as a 2 ml dose. The primary vaccination course consists of a single 2 ml dose of vaccine. The revaccination scheme consists of a single dose of 2 ml after 12 months.

In cattle, the vaccine is intended for active immunisation of naïve animals from 2 months of age or calves born to immune cattle from 3 months of age, and is administered intramuscularly as a 4 ml dose.

The primary vaccination course consists of two doses of vaccine, administered 3 weeks apart. The revaccination scheme consists of a single dose of 4 ml after 12 months.

The vaccine is indicated for use during pregnancy and lactation in both species. No data are available on safety in breeding males. According to the SPC, the vaccine should only be used in breeding males according to the benefit/risk assessment by the responsible veterinarian and/or the National Competent Authorities on the current vaccination policies against BTV.

A number of adverse reactions are described in the SPC. Vaccination may cause transient increase in rectal temperature during the first 48 hours following vaccination and local reactions at the injection site in the form of erythema, associated with mild to moderate oedema, which may persist for up to 6 days following vaccination. The oedema evolves into a painless nodule that diminishes progressively and in most cases disappears or becomes residual (≤ 1cm) before 70 days in sheep and 30 days in cattle. In rare occasions an abscess may appear.

Additionally the following adverse reactions might be observed in rare occasions in sheep and in very rare occasions in cattle: reproductive system disorders (abortion, perinatal mortality or premature parturition), systemic disorders (apathy, recumbency, prostration, fever, anorexia or lethargy). The following might be observed in very rare occasions in sheep and cattle: reduction in milk production, neurological disorders (paralysis, ataxia, blindness or incoordination), respiratory tract disorders (pulmonary congestion, dyspnoea or abnormal breathing), digestive tract disorders (rumen atony or bloating), hypersensitivity reactions (with hypersalivation), death.

No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product.

## *Safety documentation*

Sheep:

Five pivotal laboratory safety studies in sheep have been carried out in compliance with the recommendations given in the CVMP Guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) (EMA/CVMP/IWP/105506/2007). The safety studies have been carried out in the most sensitive category of the target species, using the recommended route of administration and batches of vaccine manufactured with the maximum number of strains (n=2) and containing either two times or eight times the fixed target antigen amount at the formulation step.

The laboratory safety studies presented in support of the safety of Syvazul BTV, conducted with the multi-strain vaccine Syvazul BTV-1+8 include: safety of the administration of a single dose and two repeated doses in lambs, safety of an overdose and repeat single dose in lambs. An additional supporting study conducted with mono-strain vaccine Syvazul BTV-1 is presented, evaluating safety of an overdose and repeat single dose in lambs. Two studies conducted with the multi-strain vaccine Syvazul BTV-1+8 were also carried out: one in pregnant ewes and one study in lactating ewes for the examination of the reproductive performance.

Safety of the multi-strain vaccine Syvazul BTV-1+8 in sheep in the field was investigated in three specific studies as part of a wider combined safety and efficacy field trial. These studies evaluated the safety in lambs from minimum age (3 months), in pregnant ewes and in milking ewes.

Supporting safety data from laboratory efficacy studies conducted with mono-strain and multi-strain Syvazul BTV vaccines and pharmacovigilance data available for the currently authorised vaccines have also been provided.

Cattle:

Two laboratory safety studies in cattle have been carried out in compliance with the recommendations given in the CVMP Guideline EMA/CVMP/IWP/105506/2007 on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD). The safety studies have been carried out in the most sensitive category of the target species using the recommended route of administration and batches of vaccine manufactured with the maximum number of strains (n=2) and containing either two times or eight times the fixed target antigen amount at the formulation step.

The laboratory safety studies presented in support of the safety of Syvazul BTV conducted with multi-strain vaccine Syvazul BTV-1+8 include two studies investigating safety of the administration of a single dose and two repeated doses in calves. Laboratory studies have not been carried out examining reproductive performance in cattle. Safety in pregnant and lactating cattle has been investigated as part of field studies.

Safety of the multi-strain vaccine Syvazul BTV-1+8 in cattle in the field was investigated in two specific studies as part of a wider combined safety and efficacy field trial. These studies evaluated the safety in calves from minimum age (2 months), in pregnant cows and lactating cows.

Supporting safety data from laboratory efficacy studies conducted with mono-strain and multi-strain Syvazul BTV vaccines and pharmacovigilance data available for the currently authorised vaccines have also been provided.

| ***Study reference*** | ***Study title*** | ***Batch used*** |
|---|---|---|
| Sheep studies: | | |
| EL-LA-0801i | Double dose (4 ml) and repeat single dose (2 ml) in lambs of minimum recommended age | Syvazul BTV-1+8<br>08007P<br>2x Fixed target antigen content |
| EL-LA-0801ii | Double dose (4 ml) and repeat single dose (2 ml) in lambs of minimum recommended age | Syvazul BTV-1<br>08001P<br>2x Fixed target antigen content |
| EL-LA-1201 | Three repeat single doses (2 ml) in lambs of minimum recommended age | Syvazul BTV-1+8<br>10002P<br>8x Fixed target antigen content |
| EL-LA-0803 | Three repeat single doses (2 ml) in pregnant ewes | Syvazul BTV-1+8<br>08007P<br>2x Fixed target antigen content |
| EL-LA-0902 | Two repeat single doses (2 ml) in pregnant ewes | Syvazul BTV-1+8<br>08007P |

| | | |
|---|---|---|
| | | 2x Fixed target antigen content |
| EL-LA-0805 | Three repeat single doses (2 ml) in lactating ewes | Syvazul BTV-1+8<br>08015P<br>2x Fixed target antigen content |
| SYV13-003 | Field study<br>Two repeat single doses (2 ml) in lambs from minimum recommended age | Syvazul BTV-1+8<br>150303<br>Fixed target antigen content |
| SYV13-003 | Field study<br>Two repeat single doses (2 ml) in pregnant ewes | Syvazul BTV-1+8<br>150303<br>Fixed target antigen content |
| SYV13-003 | Field study<br>Two repeat single doses (2 ml) in lactating ewes | Syvazul BTV-1+8<br>150303<br>Fixed target antigen content |
| <u>Cattle studies:</u> | | |
| EL-LA-0804 | Three repeat single doses (4 ml) in calves of minimum recommended age | Syvazul BTV-1+8<br>08007P<br>2x Fixed target antigen content |
| EL-LA-0905 | Three repeat single doses (4 ml) in calves of minimum recommended age | Syvazul BTV-1+8<br>09001P<br>8x Fixed target antigen content |
| SYV13-003 | Field study<br>Two repeat single doses (4 ml) in calves from minimum recommended age | Syvazul BTV-1+8<br>150303<br>8x Fixed target antigen content |
| EC-LA-1001 | Field study<br>Two repeat single doses (4 ml) in pregnant cows<br>Two repeat single doses (4 ml) in lactating cows | Syvazul BTV-1+8<br>10002P<br>8x Fixed target antigen content |

## *Laboratory tests*

### *Safety of the administration of one dose and repeat administration of one dose*

Sheep:

The safety of administration of one dose and the repeated administration of two single doses was investigated under laboratory conditions in one Good Laboratory Practice (GLP)-compliant study (EL-LA-1201) in which 10 lambs of the minimum recommended age were vaccinated with a total of three doses of vaccine administered, 16 and 14 days apart, by the recommended route of administration. The vaccine contained eight times the fixed target antigen amount of vaccine. Ten lambs were inoculated at the same time with a placebo. After vaccination, no systemic reactions or alteration in the health status were observed. Transient increases in rectal temperature were very commonly observed during 48 hours after first vaccination with a maximum increase of 1.8 °C, which returned to normal by 72 hours post vaccination, and 24 hours after second and third vaccination with maximum increase of 2.28 ºC, which returned to normal by 48 hours post vaccination. Local reactions at injection sites were very commonly observed in vaccinated lambs after each vaccination. These occurred as erythema/oedema from 3 days after the first and second vaccination and from 2 days after the third vaccination. These generally evolved into painless nodular swellings from 7 days after first vaccination, 4 days after second vaccination and 2 days after third vaccination, that increased in size to a maximum of 5.72 $cm^2$ (seen after first vaccination) and then regressing over time. Residual nodules of up to 1.92 $cm^2$ were seen 16 days post vaccination, and 0.56 $cm^2$ and 2.56 $cm^2$ 14 days post second and third vaccination, respectively. Histopathological examination of injection site reactions revealed self-limited granulomatous inflammation. Vaccination did not have any effect on weight gain.

In conclusion, the results show that the administration of a single dose of Syvazul BTV-1+8 and of the repeated administration of two doses of the vaccine is considered safe. Adverse reactions such as local reactions and transient increase in temperature are adequately addressed in the SPC.

Cattle:

The safety of administration of one dose and the repeated administration of two doses was investigated under laboratory conditions in two GLP-compliant studies (EL-LA-0804 and EL-LA-0905) in which 10 calves of the minimum recommended age were vaccinated with a total of three doses of vaccine, each administered 14 days apart by the recommended route of administration. Vaccine contained two and eight times the fixed target antigen amount in first and second study, respectively, and histopathological examination of injection sites was conducted in the second study only. Ten calves were inoculated at the same time with a placebo. After vaccination, no systemic reactions or alteration in the health status were observed.

In the first study, transient increases in rectal temperature were very commonly observed during 24 hours after each vaccination, which resolved by 48 hours with maximum temperature increases of 1.09 °C, 1.95 °C and 1.79 °C seen after first, second and third vaccinations, respectively. Local reactions at injection sites were not observed. Vaccination did not have any effect on weight gain.

In the second study, transient increases in rectal temperature were very commonly observed during 24–48 hours after each vaccination with maximum increases of 1.16 °C, 1.46 °C and 0.5 °C after first, second and third vaccinations, which returned to normal by 40-72 hours post vaccination. Significant differences between vaccinates and control groups were detected only at 24 hours after first

vaccination. Local reactions at injection sites were very commonly observed in calves 24 hours after first vaccination (max. 0.5 x 0.5 cm), which resolved by 48 hours, and in calves after second vaccination (max. 5 x 7 cm), which resolved by 72 hours. Vaccination did not have any effect on weight gain.

In conclusion, the results show that the administration of a single dose of Syvazul BTV-1+8 and of the repeated administration of two doses of the vaccine is considered safe. Adverse reactions such as local reactions and transient increase in temperature are adequately addressed in the SPC.

### *Safety of one administration of an overdose*

Sheep:

Although it is not currently a requirement for inactivated vaccines, the safety of the administration of a double dose (4 ml) of Syvazul BTV-1+8 in lambs, followed by administration of a single dose (2 ml) was evaluated in a GLP-compliant study (EL-LA-0801i). An additional study (EL-LA-0801ii) is provided, in which the safety of the administration of a double dose (4 ml) of Syvazul BTV-1 in lambs followed by administration of a single dose (2 ml) was evaluated. However, this latter study is considered supportive only, as it does not comply with the guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) (EMA/CVMP/IWP/105506/2007) where tests for safety should be carried out using a batch manufactured with the maximum number of strains proposed for the final product.

In study EL-LA-0801i 10 lambs of the minimum recommended age were administered with a double dose (4 ml) of vaccine by the recommended route of administration, followed 21 days later by a single dose (2 ml). The vaccine batch was formulated to contain double the fixed target antigen amount of vaccine. Ten lambs, which did not receive any treatment, were included as controls. After vaccination, no systemic reactions or alteration in the health status were observed.

Rectal temperatures of the vaccinated animals after administration of either double dose or repeat single dose were similar to the controls. Local reactions at injection sites were observed in all vaccinated lambs after each vaccination. These occurred as erythema/oedema during the first 5 days post vaccination and evolved into painless nodular swellings which increased gradually and reached maximum values (1.6 cm) from day 7 to 11 after administration of the first dose, and from day 8 to 10 after administration of the second dose and then regressed over time. Residual nodules (maximum individual nodule swelling of 0.9 cm) remained at least for 75 days after first vaccination and at least 54 days after second vaccination. One lamb developed injection site abscess in both injection sites, which eventually opened to the exterior with the consequent release of a purulent exudate. Vaccination did not have any effect on weight gain.

In conclusion, the results show that the administration of a double dose of Syvazul BTV-1+8, followed by a single dose of the vaccine, is considered safe. Adverse reactions such as local reactions are adequately addressed in the SPC.

Cattle:

Studies investing safety of administration of an overdose in cattle have not been provided. No overdose studies are required for inactivated vaccines.

## *Examination of reproductive performance*

Sheep:

The examination of reproductive performance in sheep was investigated in three different GLP-compliant laboratory studies.

In study EL-LA-0803 10 ewes in the late stage of pregnancy (100 days of gestation, approximately 4–5 weeks before lambing) were administered a total of three doses of vaccine, each administered 14 days apart, by the recommended route of administration. The vaccine batch was formulated to contain double the fixed target antigen amount of vaccine. Ten pregnant ewes that did not receive any treatment were included as controls.

After vaccination, no systemic reactions or alteration in the health status attributable to vaccination were observed. Transient slight increases in temperatures were detected (maximum individual temperature of 1.7 °C) after administration of each dose, occurring at 24 hours post vaccination, which returned to normal 48 hours post vaccination. Local reactions were observed in all the vaccinated animals after each vaccination. Reaction was initially detected as a non-measurable erythema and mild local oedema observed during the first 1-5 days after vaccination, with further evolution into measurable painless nodular swellings reaching maximum size (3 cm) at Day 7 post first vaccination, Day 5 post second vaccination, Day 3-7 (3.0 cm) post third vaccination, after which nodule size decreased, reaching minimum size (<1 cm) by Day 42 of study, with the exception of one animal with a lesion of 1.4 cm. No statistical differences were observed between vaccinated and control groups with regard to reproductive performance parameters.

Since perinatal mortality rates in both vaccinates (20%) and controls (17%) were higher than expected and attributed to excessive manipulation of pregnant sheep, a complementary study (EL-LA-0902) was conducted, in which manipulation of pregnant sheep was reduced. The design of the study was similar to the first study, with the exception that in this study 10 ewes in the late stage of pregnancy were administered a total of two doses of the vaccine 14 days apart. A control group was not included. Clinical monitoring and measurement of rectal temperatures and local reactions were not performed during the experimental period to avoid stress and physical damage associated with handling procedures, which can result in an increase in abortion rates. Post vaccination, there were no abortions and perinatal mortality rate was 7.02%, which was lower than in the previous study.

In study EL-LA-0805 10 ewes in the fourth week of lactation were administered a total of three doses of vaccine, each administered 14 days apart, by the recommended route. The vaccine batch was formulated to contain double the fixed target antigen amount of vaccine. Ten lactating ewes that did not receive any treatment were included as controls.

After vaccination, no systemic reactions or alteration in the health status attributable to vaccination were observed. Transient slight increases in temperatures were detected (maximum individual temperature of 2.2 °C) after administration of each dose, occurring at 24 hours post vaccination, which returned to normal 48 hours post vaccination. Local reactions were observed in all the vaccinated animals after each vaccination. The local reactions were initially detected as a non-measurable erythema and mild local oedema observed during the first 5 days after vaccination, with further evolution into measurable painless nodular swellings, which progressively decreased in most cases to residual lesions. Maximum size was reached at:

- 8 days after first vaccination (2.6 cm), after which nodule size decreased, and by Day 42 post

vaccination lesions were residual (<1.0 cm), with the exception of two animals, which had lesions of 1.0 and 1.1 cm.

- at 7 days after second vaccination (3.4 cm), after which nodule size decreased and by Day 42 (28 days post vaccination) residual lesions (<1.0 cm) were found in 5 animals and lesions ranging from 1.0-1.4 cm in another 5 animals, and

- between days 5-7 (3.6 cm) after third vaccination, after which nodule size decreased, reaching between 1.3-2.8 cm 14 days later, at day 42.

On the whole, the vaccination did not impact on productive parameters and statistically significant differences in body weight, milk yield, milk composition (fat, protein, lactose and dry matter) were not observed between the groups, with the exception of milk production on day 3 after administration of the first dose when average values were higher in control group animals (although a slight drop in milk production was detected after administration of both the vaccine and the placebo, returning to normal within approximately one week). There is a warning in the SPC that in very rare occasions in sheep and cattle vaccination might cause reduction in milk production.

In conclusion, the results show that the administration of a single dose of Syvazul BTV-1+8 and of the repeated administration of two doses of the vaccine is considered safe in lactating sheep. Adverse reactions such as local reactions, transient increase in temperature and the reduction in milk yield seen on day 3 post vaccination are adequately addressed in the SPC.

The examination of reproductive performance in sheep (pregnant ewes in the first and second half of pregnancy and lactating ewes) was also investigated in field studies provided in Section C using a batch of vaccine containing fixed target antigen amount.

In conclusion, results showed that no statistical differences were observed between vaccinated and control groups with regard to reproductive performance parameters in sheep and no safety concerns arose in lactating sheep.

Laboratory studies have not been carried out examining reproductive performance in cattle, with adequate justification provided that, based on the current legislation (Annex 1 Commission Directive 2009/9/EC), safety in pregnant and lactating cattle has been investigated as part of field studies in Section C.

## *Examination of immunological functions*

No specific tests on immunological functions were carried out and this is considered acceptable because Syvazul BTV is a conventional inactivated vaccine containing classical compounds with no known adverse effect on immunological functions.

## *User safety*

A user safety assessment has been conducted in accordance with the CVMP Guideline on user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006). Due to the nature and concentration of its active substances (inactivated bluetongue virus - maximum two of the following BTV serotypes: BTV-1, BTV-8 and BTV-4) and excipients (semi-purified saponin from *Quillaja saponaria*, aluminium hydroxide, silicon antifoaming agent, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate anhydrous, sodium chloride and thiomersal), the vaccine does not pose any specific risk to the user when used as recommended.

## *Study of residues*

The active substances being principles of biological origin intended to produce active immunity are not within the scope of Regulation (EC) No 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin.

## *Maximum residue limits (MRLs)*

The components of the adjuvant (saponin and aluminium hydroxide) used in the vaccine and the preservative (thiomersal) are allowed substances, for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required, whilst all the other excipients are substances considered as not falling within the scope of Regulation (EC) No 470/2009.

## *Withdrawal periods*

The withdrawal period is set at zero days.

## *Interactions*

No data has been provided investigating interactions of the vaccine with other veterinary immunological products and therefore it is proposed to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis.'

## *Field studies*

Sheep:

Safety of Syvazul BTV-1+8 in sheep in the field was investigated in three specific studies as part of a wider combined safety and efficacy field trial (SYV13-003). These studies evaluated safety in lambs from minimum age (3 months), in pregnant ewes and in milking ewes.

The safety of administration of one dose and the repeated administration of one dose was investigated in field study conducted to Good Clinical Practice (GCP) in which 35 lambs of the minimum recommended age were vaccinated with a total of two doses of vaccine administered 21 days apart by the recommended route of administration. Vaccine contained the fixed target antigen amount of vaccine. Thirty-five (35) lambs were inoculated at the same time with a placebo following the same schedule.

After vaccination, no systemic reactions or alteration in the health status attributable to vaccine were observed. Slight transient increases in rectal temperature occurred after administration of the first (mean increase of 0.4 ºC) and second (mean increase of 0.2 ºC) doses of vaccine compared to controls, occurring respectively one and two days post vaccination and returning to normal respectively two and three days post vaccination. Local reactions at injection sites were mainly detected after the second vaccination. After the first vaccination, one lamb in the vaccinate group developed swelling at injection site 1-6 days post vaccination (maximum 4.8 cm on day 3); measurable nodular induration gradually decreased from 2.4 cm at Day 8 to 0.9 cm (considered residual) by Day 64-66.

After the second vaccination, the development of nodular induration 0.5-2.6 cm from 1-6 days post vaccination was very common and resolved mostly within 10 days, with the exception of one animal in

which nodular induration gradually decreased to 1.0 cm by Day 63-65.

In conclusion, the results show that the administration of a single dose of Syvazul BTV-1+8 and of the repeated administration of one dose of the vaccine is considered safe in sheep of minimum age. Adverse reactions such as local reactions and transient increase in body temperature are adequately addressed in the SPC.

The safety of administration of one dose and the repeated administration of one dose was investigated in field study conducted to GCP in which 75 pregnant ewes in the first half of pregnancy (0–2.5 months) and 83 ewes in the second half of pregnancy (2.5–5 months) were vaccinated with a total of two doses of vaccine administered, 21 days apart, by the recommended route of administration. Vaccine contained the fixed target antigen amount of vaccine. Seventy six (76) ewes in the first half of pregnancy and 83 ewes in the second half of pregnancy were inoculated at the same time with a placebo following the same schedule. Additional sentinel controls of 47 ewes in the first half of pregnancy and 55 ewes in the second half of pregnancy were included, which were not treated.

After vaccination, no systemic reactions or alteration in the health status attributable to vaccine were observed. No significant differences were observed in any of the reproductive parameters (gestation length, proportion of heat repetitions, abortions, number of stillbirths, total birth weight and offspring survival 72 hours) between vaccinated and control group, neither when ewes were vaccinated during the first half of gestation nor when they were vaccinated during the second half.

In conclusion, the results show that the administration of a single dose of Syvazul BTV-1+8 and of the repeated administration of one dose of the vaccine is considered safe in pregnant sheep in the first and second halves of pregnancy.

The safety of administration of one dose and the repeated administration of one dose was investigated in field study conducted to GCP in which 73 lactating ewes were vaccinated with a total of two doses of vaccine administered, 21 days apart, by the recommended route of administration. Vaccine contained the fixed target antigen amount. Seventy-four (74) lactating ewes were inoculated at the same time with a placebo, following the same schedule. Additional sentinel controls of 49 lactating ewes were included, which were not treated.

After vaccination, no systemic reactions or alteration in the health status attributable to vaccine were observed. Following the first vaccination there were no statistically significant differences in milk production between any of the groups. Following the second vaccination, milk production in the vaccinated and placebo control groups were slightly higher than the sentinel controls. However, there was no statistically significant difference between milk production between ewes vaccinated with Syvazul BTV-1+8 or placebo.

In conclusion, the results show that the administration of a single dose of Syvazul BTV-1+8 and of the repeated administration of one dose of the vaccine is considered safe in lactating sheep and does not affect milk yield.

Cattle:

Safety of Syvazul BTV-1+8 in cattle in the field was investigated in two specific studies (EC-LA-1001 and SYV13-003), one of them as part of a wider combined safety and efficacy field trial (SYV13-003). These studies evaluated safety in calves from minimum age of 2 months, pregnant cows and lactating cows.

The safety of administration of Syvazul BTV-1+8 was investigated in a field study conducted to GCP in which 25 calves of the minimum recommended age were vaccinated with a total of two doses of

vaccine administered, 21 days apart, by the recommended route of administration. Vaccine contained the fixed target antigen amount. Twenty-five (25) calves were inoculated at the same time with a placebo, following the same schedule.

After vaccination, no systemic reactions or alteration in the health status attributable to vaccine were observed. There was no difference in rectal temperatures between vaccinates and controls. Local reactions at injection sites were not detected in either group. In conclusion, the results show that the administration of two single doses of Syvazul BTV-1+8 administered 21 days apart is considered safe in cattle of minimum age.

The safety of administration of Syvazul BTV-1+8 was investigated in a field study conducted to GCP in which 295 cows in different stages of pregnancy (first, second and third trimester) were vaccinated with a total of two doses of vaccine administered, 21 days apart, by the recommended route of administration. The vaccine batch was formulated to contain eight times the fixed target antigen amount. Two hundred and ninety-five cows in different stages of pregnancy (first, second and third stages) were inoculated at the same time with a placebo, following the same schedule. Additionally, 66 lactating cows were vaccinated by the same schedule and route using the same vaccine and the same number of lactating cows were inoculated at the same time with a placebo.

After vaccination, no systemic reactions or alteration in the health status attributable to vaccine were observed. No difference in rectal temperatures between vaccinates and controls were observed. Injection site reactions (pain, heat and measurable induration) occurred in vaccinated group but not in control. Local reaction scores were low (size < 1.0 cm), but statistically significant difference was found between the two groups. The duration of local reactions (size) at injection site was statistically greater in vaccinates following first vaccination than in the controls. Vaccination did not impact pregnancy parameters and no statistically significant differences were found in pregnancy length, abortion occurrence, newborn characteristics, parturition to first insemination period, and fertility between the vaccinated and control groups after administration of first or second dose of vaccine. Vaccination did not impact lactation parameters and no statistically significant differences were found in average milk production or individual daily milk production between the vaccinated and control groups after administration of first or second dose of vaccine.

In conclusion, the results show that the administration of two single doses of Syvazul BTV-1+8 administered 21 days apart is considered safe in pregnant (all trimesters) and lactating cows.

## *Supplementary safety information*

The applicant has provided supplementary safety data from 10 efficacy studies in sheep and 13 efficacy studies in cattle, conducted using mono-strain Syvazul BTV vaccines containing BTV-1 or BTV-8 and combined vaccine containing BTV-1+8. The main objective of these studies was to demonstrate efficacy, although data on general reactions, behavioural changes, and hyperthermia or injection site reactions were also recorded. These studies are not considered pivotal safety studies and most were conducted with mono-strain vaccines. The safety profiles observed support the pivotal safety studies.

## *Environmental risk assessment*

An environmental risk assessment has been provided in accordance with the CVMP Note for Guidance on the environmental risk assessment of immunological veterinary medicinal products (EMEA/CVMP/074/95). Based on the data provided, the ERA can stop at Phase I. Syvazul BTV is expected to pose a negligible risk for the environment when used according to the SPC.

### *Overall conclusions on the safety documentation*

The safety studies have been carried out using batches of vaccine manufactured with the maximum number of strains (n=2) and containing at least the fixed target antigen amount. Batches used contained fixed target antigen amount or from 2 to 8 times the fixed target antigen amount and the standardised final product. Also, the studies have been carried out in the most sensitive category of the target species using the recommended route of administration.

For sheep, the applicant has provided five pivotal laboratory studies and three specific studies of a wider field trial to investigate safety of a) the repeated administration of one dose to lambs of the minimum recommended age, pregnant ewes and lactating ewes and b) an overdose followed by a repeat single dose to lambs of the minimum recommended age.

For cattle, the applicant has provided two pivotal laboratory studies to investigate the safety of the administration of one dose followed by the repeated administration of two single doses in calves of the minimum recommended age and two specific studies of a wider field trial to investigate safety of vaccine administered by the recommended schedule to calves of minimum recommended age, pregnant cows and lactating cows.

On the basis of the results it was concluded that the safety of the targeted animals when the vaccine is administered according to the recommended schedule and via the recommended route is acceptable. The results of the safety studies have been adequately reflected in the SPC.

The safety of the vaccine has not been investigated in breeding males. Suitable warnings have been included in section 4.7 of the SPC.

Syvazul BTV is a conventional inactivated vaccine containing active substances with no known adverse effect on immunological function.

A user safety assessment in line with the relevant guideline document has been presented. Based on that assessment, the product does not pose an unacceptable risk to the user when used in accordance to the SPC.

No specific residue studies were carried out, but a withdrawal period of zero days has been justified.

No specific studies have been conducted to investigate the interactions with other veterinary medicinal products. This is duly reflected in the relevant section of the SPC.

Syvazul BTV is expected to pose a negligible risk for the environment when used according to the SPC.

# Part 4 – Efficacy

### *Introduction and general requirements*

Syvazul BTV is a multi-strain dossier vaccine containing one or two out of three different serotypes of BTV (BTV-1, strain BTV-1/ALG2006/01 E1; BTV-4, strain SPA-1/2004 and BTV-8, strain BTV-8/BEL2006/02). These BTV serotypes are considered the most relevant to the current BTV epidemiological situation in the EU.

As acknowledged in the CVMP Guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) (EMA/CVMP/IWP/105506/2007), efficacy cannot be demonstrated in the way usually required for other types of vaccines because of the number of possible combinations of antigens. This guideline indicates that mono-strain vaccines should be manufactured in compliance with the dossier for each

available master seed virus and efficacy should be shown for each of these mono-strain vaccines.

The efficacy studies presented in support of this multi-strain dossier were performed with the combinations of antigens that the applicant considers more relevant, these are: monovalent BTV-1, monovalent BTV-8, monovalent BTV-4 and bivalent BTV-1+8. Therefore, efficacy was studied for each of all the possible mono-strain vaccines in compliance with the Guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) EMA/CVMP/IWP/105506/2007 and, furthermore, for a bivalent vaccine.

This guideline also provides guidance on efficacy studies. Under section 6.3, the guideline indicates that it will be admitted that efficacy of any multi-strain vaccine containing a combination of the selected antigens (within the maximum number of antigens previously established) will be at least as efficacious as shown for each of the mono-strain vaccines. Possible known negative impact induced by certain strains should be considered. The efficacy should be demonstrated using a batch of vaccine of minimum antigen content unless there is a fixed target antigen amount at the formulation step, in each category of the target species and by the recommended route of administration. In principle, the efficacy of the vaccine should be demonstrated by a challenge in laboratory conditions for each strain.

Scientific advice was given concerning the need to perform efficacy field studies for Syvazul BTV multi-strain vaccine (which at the time could contain two different strains of the virus BTV-1 and BTV-8; BTV-4 was incorporated to the multi-strain vaccine at a later stage of the development). The scientific advice took into account the already widespread use of their serotype 1 and serotype 8 vaccines and the epidemiological situation where exposure to natural infection during a field trial would be unlikely. The applicant had stated an intention to carry out field safety studies in various animal categories of the two target species and as a result the CVMP advised to monitor the production of neutralising antibody titres in vaccinated animals as an indicator of efficacy of the vaccine during the planned safety field trials so that the antibody titres that develop in the field trials could then be compared to those observed in the corresponding laboratory trials.

## *Challenge model:*

The challenge model used for the three viruses in all the studies performed was established by the Spanish Reference Laboratory for BTV (CVL). The challenge model consisted of the inoculation by the subcutaneous route in sheep and the intravenous route in cattle of 2 ml of virulent BTV of the corresponding serotype at a concentration of $10^6$ $TCID_{50}$/ml.

For serotype 1, the challenge strain used in the efficacy studies was BTV-1/ALG 2006 01/E1 (isolated from the spleen of sheep affected by the outbreak of disease occurring 2006 in Central Algeria) and is homologous to the vaccine strain. At the time the experiments were carried out, a heterologous challenge strain was not available and the relevance of the homologous BTV-1 challenge strain is justified based on the molecular epidemiology of the virus, which demonstrates a very high genetic homology between the challenge strain and the ones isolated from the outbreaks occurring in Western Europe from 2007, showing that they all belong to a 'western' BTV group/topotype and collectively represent a western Mediterranean lineage of BTV-1. Based on this information, the relevance of the homologous BTV-1 challenge strain used in the efficacy studies with regard to the current epidemiological situation of BTV-1 in the EU is acknowledged.

For serotype 4, the challenge strain used in the efficacy studies was BTV-4/SPA2013/01 (isolated from the blood of a sheep affected during disease outbreaks in 2013 in southern Spain), which is heterologous to the vaccine strain. The relevance of the challenge strain is justified based on the

molecular epidemiology of the virus, which demonstrates a very high genetic homology between the challenge strain and the ones present in Europe from 1998 onwards, especially within the Mediterranean basin, but also in East Europe. Whilst the challenge strain and the other European isolates represent different lineages of the virus, all the European isolates clustered within a 'western' clade. In addition, it was demonstrated that strains of the different lineages are effectively neutralised by the antibodies generated by the vaccine strain and that no difference existed between *in vitro* neutralisation of the challenge strain and the strains with lower homology.

For serotype 8, the challenge strain used in the pivotal efficacy studies was BTV-8/BEL 2006 02 (isolated from the blood sample from a sheep infected during disease outbreaks in 2006 in the North and Centre of Europe), which is heterologous to the vaccine strain. The relevance of the challenge strain is based on the molecular epidemiology of the virus, which demonstrates a very high genetic homology between the challenge strain and strains present in Europe from 2006 onwards.

## *Efficacy parameters and tests:*

Viraemia was considered the primary variable to assess vaccine efficacy. Development of clinical symptoms and specific lesions were considered secondary variables, as well as levels of virus-neutralising antibodies at the moment of challenge. In addition, infectivity of the virus after challenge, detected by PCR was assessed by isolation in Vero cell cultures according to the procedure described in OIE Manual for Terrestrial Animals.

The presence of virus in blood was investigated by detection of the viral genome using either of two different reverse transcriptase polymerase chain reactions (a conventional RT-PCR assay being used in the earlier studies, which was replaced by a real-time semi-quantitative RT-qPCR assay in the later studies; the same nucleic acid extraction method being used in both assays). The assays were adequately validated for both ovine and bovine samples and shown to be fit for purpose and sufficiently sensitive, with limits of detection ranging from 0.85 to 1.32 $\log_{10}$ $TCID_{50}$/ml.

BTV-neutralising antibodies were detected by a seroneutralisation test in Vero cells. The test has been adequately validated in Laboratorios Syva to determine specific neutralising antibodies against each of the three serotypes BTV-1, BTV-4 and BTV-8 and shown to be fit for purpose.

General clinical conditions of the animals were evaluated periodically from Day 0 to 28 post-challenge by observation at the time of feeding. Clinical examination to detect specific lesions (mouth, nose, eyes and feet) was performed every 2–3 days after challenge at the time of blood collection. Rectal temperature was registered daily at least from the challenge day to Day 14 post-challenge. Clinical signs were quantified using a Clinical Reaction Index (CRI), which is a recognised scoring system for BTV efficacy studies. The scoring system is based on three different components: hyperthermia, lesions (of the mouth, nose and feet) and mortality, and scores are used to compare the rate of protection of groups within each efficacy study. Clinical signs which were associated with, or a consequence of, lesions were also quantified and included in the index (e.g. nasal discharge, oral discharge/excessive salivation and conjunctivitis/ocular discharge contributed to the scores of specific lesions in nose, mouth and eye, respectively).

## *Efficacy documentation*

In sheep, nine pivotal studies were conducted to investigate the efficacy of the product with the proposed vaccination schedule, which included eight laboratory studies and one field trial. In cattle, eleven pivotal studies were conducted to investigate the efficacy of the product with the proposed vaccination schedule, which included ten laboratory studies and one field trial.

In addition to these studies, which were conducted with the proposed vaccination schedules for the vaccine in each target species, a large number of studies have been provided with alternative vaccination schedules (e.g. a two-dose primary schedule in sheep or a one-dose primary schedule in cattle). These studies are not considered relevant to the application.

Laboratory studies were carried out in compliance with the principles of GCP, well documented and conducted in sheep and cattle of the minimum age recommended for vaccination, using pilot batches of vaccine containing the minimum antigen content fixed for the BTV strain or the same vaccine containing 32-63% of the fixed antigen content. These are referred to as "standard" and "substandard" formulated batches of vaccine, respectively, in the dossier and assessment. Production batches were used in the field trials.

| **Study reference** | **Study title** | **Batch used** |
|---|---|---|
| **Sheep studies**: | | |
| EL-LA-1002 A | OOI Syvazul BTV-1 | 10003P, 32% of the fixed BTV-1 content<br>10004P, fixed BTV-1 content |
| EL-LA-1002 B | OOI Syvazul BTV-8 | 10005P, 32% of the fixed BTV-8 content<br>10006P, fixed BTV-8 content |
| EL-LA-1402 | OOI Syvazul BTV-4 | 14001P, 50% of the fixed BTV-4 content<br>14002P, fixed BTV-4 content |
| EL-LA-0907 | OOI Syvazul BTV-1+8 | 08019P, 50% of the fixed BTV-1+8 content<br>14002P, fixed BTV-1+8 content |
| EL-LA-1001 A | DOI Syvazul BTV-1<br>Revaccination | 10003P, 32% of the fixed BTV-1 content<br>10004P, fixed BTV-1 content |
| EL-LA-1001 B | DOI Syvazul BTV-8<br>Revaccination | 10005P, 32% of the fixed BTV-8 content<br>10006P, fixed BTV-8 content |
| EL-LA-1503 | DOI Syvazul BTV-4<br>Revaccination | 14001P, 50% of the fixed BTV-4 content<br>14002P, fixed BTV-4 content |
| EL-LA-0907 | DOI Syvazul BTV-1+8<br>Revaccination | 08019P, 50% of the fixed BTV-1+8 content<br>08018P, fixed BTV-1+8 content<br>09002P, 2x fixed BTV-1+8 content<br>09003P, 4x fixed BTV-1+8 content<br>14002P, fixed BTV-1+8 content |
| SYV13-003 | Field study<br>Syvazul BTV-1 | 150301, fixed BTV-1 content |

| | | |
|---|---|---|
| | Syvazul BTV-8<br>Syvazul BTV-1+8 | 150302, fixed BTV-8 content<br>150303, fixed BTV-1+8 content |
| **Cattle studies:** | | |
| TC-0801 A | OOI Syvazul BTV-1 | 08003P, 32% of the fixed BTV-1 content |
| TC-0801B | OOI Syvazul BTV-8 | 08017P, 32% of the fixed BTV-8 content |
| EL-LA 0810 | OOI Syvazul BTV-1+8 | 08019P, 50% of the fixed BTV-1+8 content<br>08018P, fixed BTV-1+8 content |
| EL-LA 1502 | OOI Syvazul BTV-4 | 14001P, 50% of the fixed BTV-4 content<br>14002P, fixed BTV-4 content |
| TC-0801 A | DOI Syvazul BTV-1 | 08002P, 63% of the fixed BTV-1 content |
| TC-0801B | DOI Syvazul BTV-8 | 08016P, 63% of the fixed BTV-8 content |
| EL-LA 1501 | DOI Syvazul BTV-4 | 14001P, 50% of the fixed BTV-4 content<br>14002P, fixed BTV-4 content |
| EL-LA 0906 | DOI Syvazul BTV-1+8 | 08018P, fixed BTV-1+8 content |
| EL-LA-0904 A | MDA Syvazul BTV-1 | 08012P, 32% of the fixed BTV-1 content<br>08011P, 63% of the fixed BTV-1 content |
| EL-LA-0904 B | MDA Syvazul BTV-8 | 08014P, 50% of the fixed BTV-8 content<br>08013P, 63% of the fixed BTV-8 content |
| SYV13-003 | Field study<br>Syvazul BTV-1<br>Syvazul BTV-8<br>Syvazul BTV-1+8 | <br>150301, fixed BTV-1 content<br>150302, fixed BTV-8 content<br>150303, fixed BTV-1+8 content |

## *Onset of immunity*

Sheep:

Four pivotal studies have been presented to support the onset of immunity (OOI) in sheep after vaccination with the recommended primary vaccination schedule of a single 2 ml dose administered by the subcutaneous route. In these studies, OOI was investigated using a monovalent BTV-1 vaccine, a monovalent BTV-8 vaccine, a bivalent BTV-1+8 vaccine and, finally, a monovalent BTV-4 vaccine.

The OOI of a monovalent BTV-1 vaccine was investigated in a laboratory study (EL-LA-1002 A) in which two groups of 6 lambs (Assaf breed) of the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-1 strain or with the same vaccine containing 32% of the fixed antigen content. Three lambs were kept as unvaccinated controls. A total of 6 lambs of each vaccinated group and 3 control animals were challenged 39 days after the

completion of the vaccination scheme with a virulent homologous BTV-1 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. Vaccinated animals developed an immune response of SN antibodies after vaccination with the geometric mean of individual titres (GMT) at five days before challenge of 10.0 (range 5-20 for each of the vaccinated groups). The percentage of clinical protection calculated from the Clinical Reaction Index was 100% in both vaccinated groups compared to controls. In conclusion, this study is supportive of an OOI of 39 days for the monovalent BTV-1 vaccine formulated at a minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia and reduction of clinical signs and lesions.

The OOI of a monovalent BTV-8 vaccine was investigated in a laboratory study (EL-LA-1002 B) in which two groups of 6 lambs (Assaf breed) of the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-8 strain or with the same vaccine containing 32% of the fixed antigen content. Three lambs were kept as unvaccinated controls. A total of 6 lambs of each vaccinated group and 3 control animals were challenged 39 days after the completion of the vaccination scheme with a virulent heterologous BTV-8 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. Vaccinated animals developed an immune response of SN antibodies after vaccination with GMT at five days before challenge of 10.0 (range 5-20) and 11.22 (range 5-20) for each vaccinated group, respectively. The percentages of clinical protection calculated from the Clinical Reaction Index were 100% in the group vaccinated with standard batch and 97.8% in the group vaccinated with sub-standard batch compared to controls. In conclusion, this study is supportive of an OOI of 39 days for the monovalent BTV-8 vaccine formulated at a minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia and reduction of clinical signs and lesions.

The OOI of a bivalent BTV-1+8 vaccine was investigated in a laboratory study (EL-LA-0907) in which two groups of 12 lambs (Marino breed) of the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-1+8 strain or with the same vaccine containing 50% of the fixed antigen content. Six lambs were kept as unvaccinated controls. Thirty-five days after completion of the vaccination scheme, half of the lambs in each group were challenged with a homologous virulent BTV-1 strain and the other half with a heterologous virulent BTV-8 strain. The challenged animals were monitored for 4 weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge with either of the BTV strains, with the exception of one animal which was vaccinated with the substandard batch and where viraemia was detected from 3 days after challenge until the end of study post BTV-1 challenge (this lamb was negative for BTV-1-neutralising antibodies at time of challenge). In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature and clinical signs after challenge with BTV-1 or BTV-8. All vaccinated animals developed an immune response of SN

antibodies after vaccination with GMT to BTV-1 at four days before challenge of 9.4 (range 5-20) and 12.6 (range 5–40) for each vaccinated group, respectively, and to BTV-8 of 8.3 (5-20) and 9.4 (5-40), respectively. The percentages of clinical protection against BTV-1 challenge were estimated to be 99.32% and 98.68% for the standard and sub-standard vaccine, respectively, and against BTV-8 98.1% and 97.5%. In conclusion, the study is supportive of an OOI of 35 days after completion of the primary vaccination scheme for the bivalent BTV-1+8 vaccine formulated at a minimum antigenic dose proposed for each of the strains, with an efficacy claim of prevention of viraemia and reduction of clinical signs and lesions.

The OOI of a monovalent BTV-4 vaccine was investigated in a laboratory study (EL-LA-1402) in which two groups of 8 lambs (Ripollesa breed) of the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-4 strain or with the same vaccine containing 50% of the fixed antigen content (which is the reference batch for the BTV-4 potency test). Five lambs were kept as unvaccinated controls. A total of 8 lambs of each vaccinated group and 5 control animals were challenged 39 days after the completion of the vaccination scheme with a virulent heterologous BTV-4 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge, with the exception of one lamb in the group vaccinated with standard vaccine, in which a single positive result was seen on day 14 post challenge. This lamb had developed an immune response of SN after vaccination and did not show clinical signs of disease after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. All of the animals vaccinated with standard batch of vaccine developed an immune response of SN antibodies after vaccination with GMT at the time of challenge of 8 (range 5-10) compared to 6/8 (75%) of the animals vaccinated with substandard vaccine (GMT 7.0, range 5-10). The percentages of clinical protection were 100% in the group vaccinated with standard batch and 90.4% in the group vaccinated with sub-standard batch compared to controls. In conclusion, this study is supportive of an OOI of 39 days for the monovalent BTV-4 vaccine formulated at a minimum antigenic dose proposed for this strain, with an efficacy claim of reduction of viraemia and reduction of clinical signs and lesions.

Cattle:

Four pivotal studies have been presented to support the onset of immunity (OOI) in cattle after vaccination with the recommended primary vaccination schedule of two 4 ml doses administered by the intramuscular route three weeks apart. In each of these studies, OOI was investigated using, respectively, a monovalent BTV-1 vaccine, a monovalent BTV-8 vaccine, a bivalent BTV-1+8 vaccine and, finally, a monovalent BTV-4 vaccine. The applicant has also provided data with a one-dose vaccination schedule for the bivalent BTV-1+8 vaccine, but this is not considered relevant to the current application.

The OOI of a monovalent BTV-1 vaccine was investigated in a laboratory study (TC-0801 A) in which a group of 6 calves (Friesian breed) of the minimum recommended age (2 months of age), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing 32% of the fixed antigen content. The vaccine batch was produced without thiomersal or silicon antifoaming agent and adequate justification has been provided that the absence of these excipients had no effect on the potency. Two calves were kept as unvaccinated controls and were administered saline by the same schedule. All animals were challenged 21 days after the completion of

the vaccination scheme with a virulent homologous BTV-1 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. Vaccinated animals developed an immune response of SN antibodies after vaccination with GMT at the time of challenge of 142 (range 80-320). Viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. No clinical signs or lesions of bluetongue disease were detected in any of the controls or vaccinates post-challenge. Slightly higher rectal temperatures were detected in controls post challenge, which were not statistically significant. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. In conclusion, this study is supportive of an OOI of 21 days for the monovalent BTV-1 vaccine formulated at 32% of the fixed antigen content of the minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia.

The OOI of a monovalent BTV-8 vaccine was investigated in a laboratory study (TC-0801B) in which a group of 6 calves (Friesian breed) of the minimum recommended age (2 months of age), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing 32% of the fixed antigen content. The vaccine batch was produced without thiomersal or silicon antifoaming agent and adequate justification has been provided that the absence of these excipients had no effect on the potency. Two calves were kept as unvaccinated controls and were administered saline by the same schedule. All animals were challenged 21 days after the completion of the vaccination scheme with a virulent heterologous BTV-8 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. Vaccinated animals developed an immune response of SN antibodies after vaccination with GMT at the time of challenge of 70 (range 40-160). Viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. No clinical signs or lesions of bluetongue disease were detected in any of the controls or vaccinates post-challenge. A slight increase in mean rectal temperatures in control group was observed on Days 3 and 4 post-challenge. In conclusion, this study is supportive of an OOI of 21 days for the monovalent BTV-8 vaccine formulated at 32% of the fixed antigen content of the minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia.

The OOI of a bivalent BTV-1+8 vaccine was investigated in a laboratory study (EL-LA 0810) in which two groups of 12 calves (Friesian breed) of the minimum recommended age (2 months of age), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-1 and BTV-8 strains or with the same vaccine containing 50% of the fixed antigen content. Six calves were kept as unvaccinated controls and were administered saline by the same schedule. Five calves from each of the vaccinate groups and 2 calves from the control group were challenged 21 days after the completion of the vaccination scheme with either a virulent homologous BTV-1 strain and the others with a virulent heterologous BTV-8 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. Vaccinated animals developed an immune response of SN antibodies after vaccination with GMT of BTV-1 and BTV-8 antibodies at the time of challenge of 160 (range 80-320) and 65 (range 20-160), respectively, in the group vaccinated with standard antigen content and 98 (range 40-160) and 37 (range 20-80), respectively, in the group vaccinated with sub-standard antigen content. Viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. Slightly higher rectal temperatures were detected in controls post-challenge, which were not statistically significant. No clinical signs or

lesions of Bluetongue disease were detected in any of the controls or vaccinates post challenge with BTV-1. Following BTV-8 challenge, mild clinical signs were detected in the controls and in 3/5 of vaccinates that had been vaccinated with standard batch of vaccine, including mild to moderate dyspnoea, nasal discharge and lacrimation, mild lesions on mouth and nose, mild conjunctivitis. In conclusion, this study is supportive of an OOI of 21 days for the bivalent BTV1+8 vaccine formulated at 50% of the fixed antigen content of the minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia.

The OOI of a monovalent BTV-4 vaccine was investigated in a laboratory study (EL-LA 1502) in which two groups of 9 calves (Friesian Holstein crossbreed) of the minimum recommended age (8 to 10 weeks of age) were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing the fixed minimum antigen content or with the same vaccine containing 50% of the fixed antigen content (which is the reference batch for the BTV-4 potency test). Five calves were kept as unvaccinated controls and were administered saline by the same schedule. Twenty of the calves were seronegative against BTV at the beginning of the study and, in the remaining three, BTV antibodies attributable to maternally derived antibodies (MDA) were detected by ELISA on the day of vaccination (two of the three animals originally allocated to vaccinate groups were excluded and the animal allocated to the control group was seronegative by the time of challenge). Eight of the vaccinates from each group and four of the controls were challenged 21 days after the completion of the vaccination scheme with a virulent heterologous BTV-4 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. Vaccinated animals developed an immune response of SN antibodies after vaccination with GMT at the time of challenge of 31 (ranging from 20-40) and 22 (ranging from 10–40), respectively, in the group vaccinated with standard and sub-standard antigen content. Viraemia was not detected in any of the vaccinated animals after challenge, with the exception of a positive reaction in one animal in the group vaccinated with sub-standard antigen content at the first sampling point post challenge only (virus was not isolated in culture from this animal but a slightly high temperature of 39.5 ºC was observed in this animal on Day 2 post vaccination). In contrast, all the unvaccinated control animals developed viraemia after challenge. No clinical signs or lesions of bluetongue disease were detected in any of the controls or vaccinates post challenge. A slight increase in mean rectal temperatures in control group was observed on Days 2 and 4 post-challenge. In conclusion, this study is supportive of an OOI of 21 days for the monovalent BTV-4 vaccine formulated at fixed minimum antigen content proposed for this strain, with an efficacy claim of prevention of viraemia, and a reduction claim for the substandard monovalent BTV-4 vaccine formulated at 50% of the fixed minimum antigen content proposed for this strain (which is the reference batch for the BTV-4 potency test).

## *Duration of immunity*

Sheep:

Four pivotal studies have been presented to support the duration of immunity (DOI) after vaccination with the recommended primary vaccination schedule of a single 2 ml dose administered by the subcutaneous route. In each of these studies, DOI was investigated using respectively a monovalent BTV-1 vaccine, a monovalent BTV-8 vaccine, a bivalent BTV-1+8 vaccine and, finally, a monovalent BTV-4 vaccine. In these studies revaccination with a single 2 ml one year after completion of primary vaccination was also investigated.

The DOI of a monovalent BTV-1 vaccine was investigated in a laboratory study (EL-LA-1001 A) in which two groups of 10 lambs (Merino breed) of the minimum recommended age (3 months of age),

seronegative against BTV were vaccinated subcutaneously following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-1 strain or with the same vaccine containing 32% of the fixed antigen content. Seven lambs were kept as unvaccinated controls. A total of 6 lambs of each vaccinated group and 3 control animals were challenged 364 days after the completion of the vaccination scheme with a virulent homologous BTV-1 strain. The remaining, non-challenged animals were revaccinated with a single dose of the vaccine or placebo on day 364 and were used to assess the anamnestic response induced in order to confirm the efficacy of revaccination. The challenged animals were monitored for 4 weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. The percentage of clinical protection was 94.2% and 98.1% in the vaccinated groups (vaccinated with fixed target antigen amount and 32% of fixed target antigen amount, respectively) compared to controls. Vaccinated animals developed an immune response of SN antibodies after vaccination that was still detected in both vaccinated groups, with GMT at 300 days post-vaccination (60 days before challenge) of 19.97 (range 10-40) and 18.49 (range 10-40) for each vaccinated group, respectively. In conclusion, this study is supportive of a DOI of 1 year (364 days) for the monovalent BTV-1 vaccine formulated at a minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia and reduction of clinical signs and lesions. Additionally, BTV-1-neutralising titres were boosted by revaccination, demonstrating an anamnestic response and the proposed revaccination schedule of one dose of 2 ml after 12 months is supported.

The DOI of a monovalent BTV-8 vaccine was investigated in a laboratory study (EL-LA-1001 B) in which two groups of 10 lambs (Merino breed) of the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-8 strain or with the same vaccine containing 32% of the fixed antigen content. Seven lambs were kept as unvaccinated controls. A total of 6 lambs of each vaccinated group and 3 control animals were challenged 364 days after the completion of the vaccination scheme with a virulent heterologous BTV-8 strain. The remaining, non-challenged animals were revaccinated with a single dose of the vaccine or placebo on day 364 and were used to assess the anamnestic response induced in order to confirm the efficacy of revaccination. The challenged animals were monitored for 4 weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. Vaccinated animals developed an immune response of SN antibodies after vaccination that was still detected in both vaccinated groups, with GMT at 300 days post-vaccination (60 days before challenge) of 15 (range 10-40) in each vaccinated group. BTV-8-neutralising titres were boosted by revaccination, demonstrating an anamnestic response. The percentage of clinical protection was 98.5% and 96.8% in the vaccinated groups (vaccinated with fixed target antigen amount and 32% of fixed target antigen amount, respectively) compared to controls. In conclusion, this study is supportive of a DOI of 1 year (364 days) for the monovalent BTV-8 vaccine formulated at a minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia and reduction of clinical signs and lesions. Additionally, an anamnestic response was demonstrated and the proposed revaccination schedule of one dose of 2 ml after 12 months is supported.

The DOI of a bivalent BTV-1+8 vaccine was investigated in a laboratory study (EL-LA-0907) in which different groups of lambs (Merino breed) of the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended schedule with batches of vaccine containing different antigen content ranging from 0.5X (substandard), 1X (standard), 2X to 4X antigen content of a standard batch. Fourteen lambs were kept as unvaccinated controls. Groups of lambs vaccinated with substandard vaccine and controls were challenged 6 months after the completion of the vaccination scheme with a virulent homologous BTV-1 and heterologous BTV-8 strain. Groups of lambs vaccinated with standard vaccine and controls were challenged 12 months after the completion of the vaccination scheme with a virulent homologous BTV-1 and heterologous BTV-8 strain. The remaining, non-challenged animals were revaccinated with a single dose of the vaccine or placebo on day 360 and were used to assess the anamnestic response induced in order to confirm the efficacy of revaccination. The challenged animals were monitored for 4 weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. BTV-8-neutralising titres were boosted by revaccination, demonstrating an anamnestic response. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. Vaccinated animals developed an immune response of SN antibodies after vaccination with titres at 300 days post-vaccination (60 days before challenge) ranging from 10-20 and to BTV-8 with titres ranging from 20-40. BTV-1- and BTV-8-neutralising titres were boosted by revaccination, demonstrating an anamnestic response. The percentage of clinical protection in the group vaccinated with standard vaccine was estimated to be 100% compared to control against BTV-1 and BTV-8 challenge. In conclusion, this study is supportive of a DOI of 1 year (360 days) for the bivalent BTV-1+8 vaccine formulated at a minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia and reduction of clinical signs and lesions. Additionally, an anamnestic response was demonstrated and the proposed revaccination schedule of one dose of 2 ml after 12 months is supported.

The DOI of a monovalent BTV-4 vaccine was investigated in a laboratory study (EL-LA-1503) in which two groups of 20 lambs (Laucane breed) of the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-4 strain or with the same vaccine containing 50% of the fixed antigen content (which is the reference batch for the BTV-4 potency test). Eight lambs were kept as unvaccinated controls. A total of 8 lambs of each vaccinated group and 4 control animals were challenged 360 days after the completion of the vaccination scheme with a virulent heterologous BTV-4 strain. The remaining, non-challenged animals were revaccinated with a single dose of the vaccine or placebo on Day 360 and were used to assess the anamnestic response induced in order to confirm the efficacy of revaccination. The challenged animals were monitored for 4 weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge, with the exception of one animal vaccinated with the substandard formulated batch of vaccine containing 50% of the fixed antigen content (which is the proposed reference batch of BTV-4 vaccine used in the potency test to batch release final product) and from which the virus was isolated by culture (neutralising antibody immune response in this animal was low and was detected only at one sample point Day 42 post vaccination). In contrast, all the unvaccinated control animals developed viraemia after challenge. In addition, vaccination significantly reduced the rise of rectal temperature observed after challenge. Whilst 7/32 of the vaccinates (4/16 vaccinated with sub-standard vaccine and 3/16 vaccinated with standard vaccine) did not have detectable BTV-4 seroneutralising antibodies at 42 days post

-vaccination, all had detectable antibodies from 70 days post vaccination, with levels remaining reasonably stable until challenge. BTV-4-neutralising titres were boosted by revaccination, demonstrating an anamnestic response. The percentage of clinical protection was 98.65% and 95.95% in the vaccinated groups (vaccinated with fixed target antigen amount and 50% of fixed target antigen amount, respectively) compared to controls. In conclusion, the results of this study would be supportive of 12-month DOI for the monovalent BTV-4 vaccine formulated at the proposed minimum antigenic dose with an efficacy claim of prevention of viraemia and reduction of clinical signs and lesions. An efficacy claim of reduction of viraemia and clinical signs and lesions is supported for the substandard monovalent BTV-4 vaccine formulated at 50% of the fixed minimum antigen content proposed for this strain (which is the reference batch for the BTV-4 potency test). Additionally, an anamnestic response was demonstrated and the proposed revaccination schedule of one dose of 2 ml after 12 months is supported.

Cattle:

Four pivotal studies have been presented to support the duration of immunity (DOI) after vaccination with the recommended primary vaccination schedule of two 4 ml doses administered by the intramuscular route three weeks apart. In each of these studies, DOI was investigated using respectively a monovalent BTV-1 vaccine, a monovalent BTV-8 vaccine, a bivalent BTV-1+8 vaccine and, finally, a monovalent BTV-4 vaccine. Revaccination with a single 4 ml one year after completion of primary vaccination was also investigated in the studies with the monovalent BTV-4 vaccine and the bivalent BTV-1+8 vaccine.

The DOI of a monovalent BTV-1 vaccine was investigated in a laboratory study (TC-0801 A) in which a group of 6 calves (Friesian breed) of the minimum recommended age (7-10 weeks of age), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing 63% of the fixed antigen content. The vaccine batch was produced without thiomersal or silicon antifoaming agent and adequate justification has been provided that the absence of these excipients had no effect on potency. Two calves were kept as unvaccinated controls and were administered saline by the same schedule (one of these calves was enrolled ten months after vaccination to adjust the number of control animals for challenge 12 months after vaccination and with the exception of being younger, all other characteristics of the new subject were similar to the ones beginning the study). All animals were challenged 362 days after the completion of the vaccination scheme with a virulent homologous BTV-1 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. Vaccinated animals developed an immune response of SN antibodies after vaccination with GMT of 92 (range 40-160) 362 days post vaccination at the time of challenge. Viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. No clinical signs or lesions of bluetongue disease were detected in any of the controls or vaccinates post challenge. Slightly higher rectal temperatures were detected in controls 3 days post challenge than in vaccinates. In conclusion, this study is supportive of a DOI of 362 days for the monovalent BTV-1 vaccine formulated at 63% of the fixed antigen content of the minimum antigenic dose proposed for this strain, with an efficacy claim of prevention of viraemia.

The DOI of a monovalent BTV-8 vaccine was investigated in a laboratory study (TC-0801B) in which a group of 5 calves (Friesian breed) of the minimum recommended age (7-10 weeks of age), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing 63% of the fixed antigen content. The vaccine batch was produced without thiomersal or silicon antifoaming agent and adequate justification has been provided that the absence of

these excipients had no effect on potency. Two calves were kept as unvaccinated controls and were administered saline by the same schedule (these calves were enrolled ten months after vaccination, to be used as control animals for challenge 12 months after vaccination and with the exception of being younger, all other characteristics of the new subjects were similar to the ones beginning the study). All animals were challenged 362 days after the completion of the vaccination scheme with a virulent heterologous BTV-8 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. Vaccinated animals developed an immune response of SN antibodies after vaccination with GMT of 61 (range 20-160) 362 days post vaccination at the time of challenge. Viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. No clinical signs or lesions of bluetongue disease were detected in any of the controls or vaccinates post challenge. In conclusion, this study is supportive of a DOI of 362 days for the monovalent BTV-8 vaccine formulated at 63% of the fixed antigen content of the minimum antigenic dose proposed for this strain with an efficacy claim of prevention of viraemia.

The DOI of a bivalent BTV-1+8 vaccine was investigated in a laboratory study (EL-LA 0906) in which a group of 21 calves (Friesian breed) of the minimum recommended age (10-12 weeks of age), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-1 and BTV-8 strains. Ten calves were kept as unvaccinated controls and were administered saline by the same schedule. Three hundred and sixty-four days post vaccination groups of 5 calves from the vaccinate group and 2 controls were either challenged with a virulent homologous BTV-1 or heterologous BTV-8. The remaining, non-challenged animals were revaccinated with a single dose of the vaccine or placebo on Day 364 and were used to assess the anamnestic response induced in order to confirm the efficacy of revaccination. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of seroneutralising (SN) antibodies against BTV. Vaccinated animals developed an immune response of SN antibodies from 21 days after completion of primary vaccination and at the time of challenge at day 364 cattle vaccinated with BTV-1 vaccine had titres ranging from 20-80 and with BTV-8 vaccine titres ranging from 20-40. Viraemia was not detected in any of the vaccinated animals after challenge with either BTV-1 or BTV-8. In contrast, all the unvaccinated control animals developed viraemia after challenge. No clinical signs or lesions of bluetongue disease were detected in any of the controls or vaccinates post challenge. Revaccination of animals with a single 4 ml dose of vaccine a year after primary vaccination was shown to result in an increase in neutralising antibody titres to levels greater than that seen after completion of primary vaccination. In conclusion, this study is supportive of a DOI of 364 days for the bivalent BTV-1+8 vaccine containing the minimum antigen content fixed for the BTV-1 and BTV-8 strains, with an efficacy claim of prevention of viraemia. Additionally, revaccination of animals with a single 4 ml dose of vaccine a year after primary vaccination was shown to result in an increase in neutralising antibody titres to levels greater than that seen after completion of primary vaccination.

The DOI of a monovalent BTV-4 vaccine was investigated in a laboratory study (EL-LA 1501) in which two groups of 13 calves (Friesian Holstein cross-breed) of the minimum recommended age (8–10 weeks), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing the minimum antigen content fixed for the BTV-4 strain or with the same vaccine containing 50% of the fixed antigen content (which is the reference batch for the BTV-4 potency test). Seven calves were kept as unvaccinated controls. At the beginning of the study, BTV antibodies attributed to the presence of MDA were detected by ELISA in 12 of the calves. The ELISA-positive calves that were allocated to control groups progressively lost positive result. None of

the calves presented detectable BTV-4-neutralising antibodies at vaccination day. A total of seven calves of each vaccinated group and 4 control animals were challenged 365 days after the completion of the vaccination scheme with a virulent heterologous BTV-4 strain. The remaining, non-challenged animals were revaccinated with a single dose of the vaccine or placebo on day 365 and were used to assess the anamnestic response induced in order to confirm the efficacy of revaccination. The challenged animals were monitored for 4 weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. Vaccination was shown to prevent viraemia after challenge in all but one vaccinate with sub-standard formulated batch of vaccine (which is the proposed reference batch of BTV-4 vaccine used in the potency test to batch release final product), where viraemia was detected by RT-PCR continuously from Days 2–28 post challenge (and from which virus was isolated by culture) and in all but one vaccinate with standard vaccine, in which a non-conclusive result was detected at Days 2 and 4 post challenge (and from which virus could not be isolated by culture). In contrast, BTV genome was detected in all the control animals from Days 2 to 28, threshold cycle values peaking around day 8. All vaccinated animals developed BTV-4 seroneutralising antibodies after vaccination: antibodies were detected in all vaccinated animals from Day 42 (21 days after completion of primary vaccination schedule) ranging from 5-160 (GMT 40) and 10–320 (GMT 32) in the groups vaccinated with sub-standard and standard batches of vaccine, respectively, and at time of challenge (at Day 365 post vaccination) BTV-4 seroneutralising antibodies were detected in all animals challenged ranging from 5-20 (GMT 8) and 5-20 (GMT 11) in the respective groups. No clinical signs or lesions of bluetongue disease were detected in any of the controls or vaccinates post challenge. Mean rectal temperatures were slightly higher in the control group than the vaccinated groups, but this was not statistically significant. Revaccination of animals with a single 4 ml dose of vaccine a year after primary vaccination was shown to result in an increase in neutralising antibody titres to levels greater than that seen after completion of primary vaccination. In conclusion, this study is supportive of a DOI of 365 days for the monovalent BTV-4 vaccine formulated with either 100% or 50% of the fixed antigen content of the minimum antigenic dose proposed for this strain, with an efficacy claim of reduction of viraemia.

## *Maternally derived antibodies (MDA)*

Sheep:

The influence of MDA on the efficacy of Syvazul BTV-1 and BTV-8 in sheep was investigated in two studies in which the vaccination schedule consisted of 2 doses, which is different to the one dose proposed by the applicant. As a result, these studies were not considered relevant for this application. The influence of MDA on the efficacy of Syvazul BTV-4 was not investigated. In the absence of relevant studies and robust justification regarding the influence of MDA on the efficacy of Syvazul BTV a suitable warning has been included in the SPC that no information is available on the use of the vaccine in animals with maternally derived antibodies.

Cattle:

The influence of MDA on the efficacy of Syvazul BTV in cattle was investigated in two studies: in one study cattle with MDA were vaccinated with the mono-strain vaccine Syvazul BTV-1 and in the other study cattle with MDA were vaccinated with the mono-strain vaccine Syvazul BTV-8. In both studies the vaccine was administered to cattle following the proposed vaccination schedule of 2 doses of 4 ml of vaccine given by the intramuscular route 3 weeks apart and challenged 21 days after receiving the second dose of vaccine.

The influence of MDA on efficacy of a monovalent BTV-1 vaccine was investigated in a laboratory study (EL-LA-0904 A) in which two groups of 6 calves (Friesian breed) of the minimum recommended age

(aged 12.3 to 14 weeks at vaccination), born to mothers from a BTV disease-free area that had been vaccinated against BTV-1 and presenting different levels of MDA passively acquired via colostrum, were vaccinated intramuscularly. Vaccination followed the recommended schedule with batches of vaccine containing the 63% or 32% of the fixed minimum antigen content of a standard batch. Two calves were kept as unvaccinated controls and were administered saline by the same schedule. All animals were challenged 21 days after the completion of the vaccination scheme with a virulent homologous BTV-1 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. All animals included on the study displayed MDA acquired via colostrum ingestion at the beginning of the study when aged 4.6 to 6.3 weeks ranging from 20–80 (GMT 35.6) and 10-40 (GMT 17.8) in the calves allocated to the vaccinate groups and 5-10 (GMT 7.1) in the two calves in the control group. Titres had decreased by day of first vaccination (Day 0) when the calves were 12–14 weeks of age and were only detectable in 4/10 of the calves in the groups to be vaccinated (ranging from 5–10 with GMT of 7.1 and 5) and 1/2 of the controls (GMT 5). Three weeks post vaccination titres had increased in both vaccinate groups, ranging from 80–320 (GMT 121.3 and 183.8, respectively). In contrast, neutralising antibody titres were not detected in the control animals post vaccination. Statistical difference was detected between the control and vaccinate groups. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. Only very mild clinical signs were detected in vaccinated or control animals after challenge, consisting of mild conjunctivitis or eye discharge. The study was not designed in complete compliance with the CVMP Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals (EMA/CVMP/IWP/439467/2007), as it lacked a vaccinated MDA-negative group. However, to address this, supporting data have been provided from the relevant group of Syvazul BTV-1 vaccinated animals in the OOI/DOI Study TC-0801A, which was acceptable. These calves were MDA-negative and vaccinated aged 7 to 10 weeks (mean 8.5 weeks). Neutralising antibody titres 21 days after completion of primary vaccination between the groups in the two studies were not significantly different.

In conclusion, Study EL-LA-0904 A (BTV-1 in cattle) provides evidence that vaccination with Syvazul BTV-1 of three-month-old calves with MDA to BTV-1 (born to dams vaccinated with two doses of Syvazul BTV-1 and BTV-8 in the first and second trimester of pregnancy) were protected against challenge with virulent BTV-1. Whilst the numbers of animals with MDA in the study was low (only 40% i.e. 4/10 of the animals had detectable MDA at time of vaccination), in the context of the justification provided that the antibody levels found in calves at time of vaccination are representative of those found in the field in calves, the rapporteur considers supported the proposed indication for use of vaccine containing BTV-1 antigen from 3 months of age in calves born to immune cattle.

The influence of MDA on efficacy of a monovalent BTV-8 vaccine was investigated in a laboratory study (EL-LA-0904 B) in which two groups of 6 calves (Friesian breed) of the minimum recommended age (aged 11.9 to 14.1 weeks at vaccination), born to mothers from a BTV disease-free area that had been vaccinated against BTV-8 and presenting different levels of MDA passively acquired via colostrum, were vaccinated intramuscularly following the recommended schedule with a batches of vaccine containing the 63% or 32% of the fixed minimum antigen content of a standard batch. Two calves were kept as unvaccinated controls and were administered saline by the same schedule. All animals were challenged 21 days after the completion of the vaccination scheme with a virulent heterologous BTV-8 strain. The challenged animals were monitored for four weeks after challenge for the presence of viraemia (by real-time RT-PCR), clinical signs associated with BTV infection and the presence of SN antibodies against BTV. All animals included on the study displayed MDA acquired via colostrum ingestion at the beginning of the study when calves were 4.15 to 6.4 weeks old, ranging from 10-40 (GMT 20 and GMT

17.8, respectively) in the calves allocated to the vaccinate groups and 5-10 (GMT 7.9) in the two calves in the control group. Titres had decreased by day of first vaccination (Day 0) when the calves were 12–14 weeks of age, and were only detectable in 3/10 of the calves in the groups to be vaccinated (ranging from 5–10 with GMT of 5.0 and 7.1) and 1/3 of the controls (GMT 5). Three weeks post vaccination titres had increased in both vaccinate groups, ranging from 40–80 (GMT 60.6) and 40–160 (GMT 80) in the sub-standard and standard groups respectively. In contrast, neutralising antibody titres were not detected in the control animals post vaccination. The results of the study showed that viraemia was not detected in any of the vaccinated animals after challenge. In contrast, all the unvaccinated control animals developed viraemia after challenge. Only very mild clinical signs were detected in vaccinated or control animals after challenge, consisting of mild conjunctivitis or eye discharge. The study was not designed in complete compliance with EMA/CVMP/IWP/439467/2007 (Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals) in that it lacked a vaccinated MDA-negative group. Some supporting data have been provided to address this from the relevant group of Syvazul BTV-8-vaccinated animals in OOI/DOI Study TC-0801B, which was considered acceptable. These calves were MDA-negative and vaccinated aged 7 to 9.5 weeks (mean 8.5 weeks). Neutralising antibody titres 21 days after completion of primary vaccination between the groups in the two studies were not significantly different. In conclusion, Study EL-LA-0904 B (BTV-8 in cattle) provides evidence that vaccination with Syvazul BTV-8 of three-month-old calves with MDA to BTV-8 (born to dams vaccinated with two doses of Syvazul BTV-1 and BTV-8 in the first and second trimester of pregnancy) were protected against challenge with virulent BTV-8. Whilst the numbers of animals with MDA in the study was low (only 30% i.e. of the animals had detectable MDA at time of vaccination), in the context of the justification provided that the antibody levels found in calves at time of vaccination are representative of those found in the field in calves, the rapporteur considers supported the proposed indication for use of vaccine containing BTV-8 antigen from 3 months of age in calves born to immune cattle.

The influence of MDA on efficacy of BTV-4 has not been investigated and therefore a warning has been included in section 4.4 of the SPC that no information is available on the use of the vaccine containing BTV-4 serotype in cattle with maternally derived antibodies.

## *Field trials*

Scientific advice was provided by EMA (EMA/CVMP/SAWP/434002/2013) in response to a question put by the applicant about the need to perform efficacy field studies for Syvazul BTV, taking into account the already widespread use of their serotype 1 and serotype 8 vaccines and the epidemiological situation where exposure to natural infection during a field trial would be unlikely. The CVMP advised that neutralising antibody titres can be used as a surrogate indicator of protection in field studies. Antibody titres that develop in the field trials could then be compared to the titres observed in the corresponding laboratory trials. In line with the above efficacy in the field of Syvazul BTV-1, Syvazul BTV-8 and the combined vaccine Syvazul BTV-1+8 in sheep and cattle was investigated as part of a wider combined safety and efficacy trial (SYV13-003) where efficacy was assessed using as main indicator the neutralising antibody titres developed after vaccination and was compared to that observed in the corresponding laboratory trials.

Sheep:

Efficacy in the field of the monovalent BTV-1 and BTV-8 and the bivalent BTV-1+8 vaccines was investigated in a study as part of a wider combined safety and efficacy trial (SYV13-003), conducted at a commercial sheep farm in which groups of 35 lambs from the minimum recommended age (3 months of age), seronegative against BTV were vaccinated subcutaneously following the recommended

schedule with a batch of vaccine containing the standard antigen content fixed for each of the strains. Thirty-five lambs were kept as unvaccinated controls and were administered saline. Efficacy was assessed by comparing the BTV-neutralising antibody titre responses at Days 35 and 63 post vaccination, with thresholds estimated from corresponding relevant laboratory studies. Non-inferiority of antibody titres developed by animals in the field was compared to the lowest mean of antibody titres developed by groups of animals that were protected by challenge in the relevant laboratory studies (the lower limit of the 95% confidence interval of the mean is higher than the mean threshold determined in the laboratory study). The serology data showed that the means in the field study were not inferior to those of laboratory studies: antibody titres at Day 63 post vaccination for each of the group remained at levels similar to the Day 35 post vaccination and above threshold levels calculated from the laboratory studies.

Cattle:

Efficacy in the field of the monovalent BTV-1 and BTV-8 and the bivalent BTV-1+8 vaccines was investigated in a study as part of a wider combined safety and efficacy trial (SYV13-003) in which groups of 25 calves from the minimum recommended age (2 months of age), seronegative against BTV were vaccinated intramuscularly following the recommended schedule with a batch of vaccine containing the standard antigen content fixed for each of the strains. Twenty-five lambs were kept as unvaccinated controls and were administered saline. Efficacy was assessed by comparing the BTV-neutralising antibody titre responses at Days 42 and 63 (21 and 42 days after completion of the primary vaccination schedule), with thresholds from corresponding laboratory studies. Non-inferiority of antibody titres developed by animals in the field was compared to the lowest mean of antibody titres developed by groups of animals that were protected by challenge in the relevant laboratory studies. The serology data showed that antibody titres at Day 63 post vaccination for each of the groups remained at levels similar to the Day 42 post vaccination and above threshold levels calculated from the laboratory studies.

Efficacy field data for Syvazul BTV-4 have not been provided in either target species. This lack of field efficacy data for the BTV-4 serotype presents a data gap since it is a requirement of the multi-strain guideline that, unless justified, results from laboratory studies should be supplemented with data from field trials. The laboratory efficacy studies conducted with each of the Syvazul BTV mono-strain vaccines (BTV-1, BTV-4, BTV-8) have shown serological immune responses in both target species following vaccination as demonstrated by the production of seroneutralising antibody titres. In the case of the mono-strain BTV-1 and BTV-8 vaccines, efficacy demonstrated in the laboratory studies was supplemented by field studies, where serological immune responses where collected and compared to those observed in the laboratory studies (mean seroneutralising antibody titres in the respective field studies were not inferior to those of the laboratory studies). The lack of field data for BTV-4 means that serology data from the field are not available to supplement the results of the laboratory studies. Nevertheless, whilst the existing gap in data provided for this serotype (serology data only available for seronegative animals) was acknowledged, it was considered that the available efficacy data are sufficient to support the proposed indication for the BTV-4 serotype in both target species.

## *Overall conclusion on efficacy*

The efficacy of Syvazul BTV has been demonstrated under laboratory conditions where sheep and cattle were vaccinated according to the recommended vaccination schedule and challenged with a virulent homologous BTV strain in the case of BTV-1 and a heterologous BTV strain in the case of BTV-4 and BTV-8 after a defined period of time.

The onset and duration of immunity studies have been carried out including animals of the minimum

recommended age of the target species and using batches of vaccine containing the minimum antigen content fixed for the BTV strain or the same vaccine containing 32–63% of the fixed antigen content.

In sheep, an OOI of 39 days after completion of the primary vaccination scheme with a DOI of 12 months were demonstrated for the monovalent vaccines containing BTV-1, BTV-4 or BTV-8 and for the bivalent vaccine containing BTV-1 and BTV-8. The efficacy claim supported by these studies is prevention of viraemia and reduction of clinical signs and lesions caused by serotypes BTV-1 and BTV-8 and reduction of viraemia and clinical signs and lesions caused by serotype BTV-4 (for BTV-4 a reduction of viraemia claim is supported: reduction of viraemia was demonstrated at OOI and prevention was demonstrated at DOI).

In cattle, an OOI of 21 days after completion of the primary vaccination scheme with a DOI of 12 months were demonstrated for the monovalent vaccines containing BTV-1, BTV-4 or BTV-8 and for the bivalent vaccine containing BTV-1 and BTV-8. The efficacy claim supported by these studies is prevention of viraemia caused by BTV-1 and BTV-8 and reduction of viraemia caused by BTV-4 (for BTV-4 a reduction of viraemia claim is supported: whilst prevention of viraemia was demonstrated at OOI, reduction of viraemia was demonstrated at DOI).

The proposed single-dose annual revaccination schedule for both target species has been demonstrated with revaccination resulting in an increase in neutralising antibody titres greater than those seen after completion of primary vaccination.

The influence of MDA on the efficacy of Syvazul BTV in sheep has not been investigated in relevant studies and a suitable warning has been included in the SPC. Efficacy of vaccine containing BTV-1 or BTV-8 administered from 3 months of age in calves born to immune cattle has been demonstrated. However, no data have been provided on the influence of MDA in cattle vaccinated with BTV-4 and therefore a suitable warning has been included in the SPC.

Efficacy in the field of Syvazul BTV-1, Syvazul BTV-8 and the combined vaccine Syvazul BTV-1+8 in sheep and cattle has been demonstrated. However, efficacy field data for Syvazul BTV-4 have not been provided in either target species. This lack of field efficacy data for the BTV-4 serotype presents a data gap. However, it is considered that the available data are sufficient to support the proposed indication for the BTV-4 serotype in both target species.

# Part 5 – Benefit-risk assessment

## *Introduction*

Syvazul BTV is an inactivated, bluetongue vaccine consisting of 1-2 virus(es) out of a set of 3 viruses for active immunisation of sheep and cattle against BTV serotypes 1, 4 and 8. The active substances are the inactivated BTV serotypes 1, 4 and 8. The vaccine contains aluminium hydroxide and saponin as adjuvants, thiomersal as preservative.

Syvazul BTV is a multi-strain dossier application.

The vaccine is presented as a suspension for injection to be administered by subcutaneous route in sheep and intramuscular route in cattle.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

## *Benefit assessment*

## *Direct therapeutic benefit*

In well-conducted laboratory and field studies, Syvazul BTV was shown to induce active immunisation in sheep from 3 months of age for the prevention of viraemia and reduction of clinical signs and lesions caused by bluetongue virus, serotypes 1 and 8 and reduction of viraemia and clinical signs and lesions caused by bluetongue virus, serotype 4. An overall onset of immunity of 39 days for all strains, with duration of immunity for 12 months, is supported.

In well-conducted laboratory and field studies, Syvazul BTV was shown to induce active immunisation in cattle from 2 months of age for the prevention of viraemia caused by bluetongue virus, serotypes 1 and 8 and reduction of viraemia caused by bluetongue virus, serotype 4. An overall onset of immunity of 21 days for all strains, with duration of immunity for 12 months, is supported.

Revaccination 12 months after completion of primary vaccination schedule has been demonstrated in both target species.

## *Additional benefits*

The ability to mix different BTV strains (up to two) gives flexibility to react to emergency bluetongue situations. Vaccines against bluetongue virus (BTV) represent a special case in terms of the need for rapid and frequent change in the serotypes included in the vaccines. This is due to the unpredictability of the virus incursions and outbreaks of disease and the number of different serotypes of BTV that exist. The strains included are relevant to the current epidemiological situation of BTV in the EU.

## *Risk assessment*

Main potential risks have been identified as follows:

*Quality*:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

*Risks for the target animal*:

The product is generally well tolerated in the target animals. The most common adverse reactions are the development of local reactions at the site of injection after vaccination, erythema associated with mild to moderate oedema and painless nodules, and a transient increase in rectal temperature following vaccination.

Appropriate warnings are included in the SPC.

The influence of maternal antibodies on the efficacy of the vaccine in sheep has not been investigated and suitable warnings have been included in the SPC. The influence of BTV-4 specific maternal antibodies on the efficacy of the vaccine in cattle has not been investigated and a suitable warning has been included in the SPC.

The safety of the vaccine has not been investigated in breeding males of either target species and suitable warnings have been included in the SPC.

*Risk for the user*:

The potential risks to the person administering the product, as well as other persons in direct contact with the animals have been evaluated in relation with the components of Syvazul BTV. The CVMP concluded that the user safety profile for this product is acceptable when used according to the SPC recommendations.

*Risk for the environment*:

The product is not expected to pose any risk to the environment when used as recommended.

*Risk for the consumer*:

The adjuvants and excipients listed 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 considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this veterinary medicinal product.

## *Risk management or mitigation measures*

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target species, the user, the consumer and the environment, and to provide advice on how to prevent or reduce these risks.

## *Evaluation of the benefit-risk balance*

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

## *Conclusion*

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Syvazul BTV is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.