15 January 2025
EMA/20636/2025
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Syvazul BTV 3 (EMEA/V/C/006623/0000)

Vaccine common name: Bluetongue virus vaccine (inactivated)

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. submitted on 1 October 2024 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Syvazul BTV 3, through the centralised procedure under Article 42(2)(c) of Regulation (EU) 2019/6 (**mandatory scope**).

The eligibility to the centralised procedure was agreed upon by the CVMP on 19 June 2024 as Syvazul BTV 3 contains an active substance which has not been authorised as a veterinary medicinal product within the Union at the date of the submission of the application (Article 42(2)(c)).

Syvazul BTV 3 is currently in use in the Netherlands, Belgium, Germany, Austria, Spain and Portugal under Article 110 of Regulation (EU) 2019/6.

At the time of submission, the applicant applied for the following indications:

"Sheep: for active immunisation of sheep to reduce viraemia, to prevent mortality and reduce clinical signs and lesions caused by bluetongue serotype 3.

Cattle: for active immunisation against bluetongue virus serotype 3."

The active substance of Syvazul BTV 3 is Bluetongue virus, serotype 3, BTV-3/NET2023, inactivated, and contains two adjuvants (Aluminium hydroxide and Purified saponin (Quil-A) from *Quillaja saponaria*). The proposed target species are Cattle and Sheep. The route of administration is subcutaneous in sheep and intramuscular in cattle.

Syvazul BTV 3 is a suspension for injection containing ≥ $10^{6.9}$ $CCID_{50}$ (the ($CCID_{50}$) 50 % cell culture infective dose determined before inactivation) of Bluetongue virus, serotype 3, BTV-3/NET2023, inactivated and is presented in cardboard box with one polypropylene colourless vial containing 80 ml or 200 ml.

The manufacturing of Syvazul BTV 3 follows the same process as that used for the centrally authorised multi-strain vaccine, Syvazul BTV.

The rapporteur appointed is Rory Breathnach and the co-rapporteur is Jacqueline Poot.

The dossier has been submitted in line with the requirements for submissions under Article 25 of Regulation (EU) 2019/6 – application in exceptional circumstances.

For the assessment of this procedure, an accelerated timetable was applied for by the applicant and agreed by the CVMP. In fact, the benefit of the immediate availability on the market of a veterinary medicinal product against BTV virus serotype 3, currently circulating in the European Union (EU), was recognised by the CVMP.

On 15 January 2025, the CVMP adopted an opinion and CVMP assessment report.

On 20 February 2025, the European Commission adopted a Commission Decision granting the marketing authorisation for Syvazul BTV 3.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

The applicant has provided a summary of the pharmacovigilance system master file which fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided, the applicant has in place a pharmacovigilance system master file (PSMF), has the services of a qualified person responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

Manufacturing authorisations and inspection status

Active substance

Manufacture of the active substance Bluetongue virus, serotype 3, BTV-3/NET2023, Inactivated takes place at Laboratorios Syva S.A., Spain.

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 audit by the manufacturing site responsible for batch release which has taken into consideration the GMP certificate available for the active substance site issued by the Spanish competent authority following inspection.

Finished product

Batch release of the finished product takes place at Laboratorios Syva S.A., Parque Technologico De Leon, Calle Nicostrato Vela M15-M16, Leon, Spain. Other activities also take place in another site in Spain.

The Laboratorios Syva site has a manufacturing authorisation issued on 22/12/2020 by the Spanish Competent Authority.

GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture of such veterinary dosage forms, has been provided for the Laboratorios Syva site. A GMP certificate for the other site, which confirms the date of the last inspection and shows that the site is authorised, is available on EudraGMP.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file is considered to be in line with legal requirements.

The GMP status of the active substance and of the finished product manufacturing site has been satisfactorily established and is in line with legal requirements.

The applicant adequately summarised the documentation submitted and provided a critical expert report in part 1c which included justification for the major data gaps. Additionally, the applicant argued in favour of the benefits of the immediate availability on the market of Syvazul BTV 3.

Part 2 - Quality

Quality documentation (physico-chemical, biological, and microbiological information)

Qualitative and quantitative composition

Syvazul BTV 3 is an inactivated vaccine presented as a ready to use liquid containing strain BTV-3/NET2023 from serotype 3 of bluetongue virus (BTV) as active substance at a titre of $\geq 10^{6.9}$ $CCID_{50}$/ml. The product contains purified saponin (Quil A) and aluminium hydroxide as adjuvant.

Other ingredients are silicone antifoam and phosphate buffer solution (PBS).

The vaccine is intended to be available in multidose presentations and consequently contains thiomersal as a preservative.

The product is available in polypropylene flasks of a 100 ml or 250 ml nominal volume filled with 80 ml or 200 ml respectively, a bromobutyl rubber stopper and an aluminium cap in a cardboard box as described in section 5.4 of the SPC.

The pack sizes are consistent with the dosage regimen and duration of use.

Container and closure system

The vaccine is filled into 100 ml or 250 ml nominal capacity polypropylene flasks (in accordance with Ph. Eur. chapters 3.1.3 and 3.1.6) containing 50 and 200 doses respectively. These are closed with bromobutyl rubber stoppers (type 1 in accordance with Ph. Eur. chapter 3.2.9) and aluminium seals.

The containers and closures are in compliance with the pharmacopoeial requirements and are adequately sterilised.

Product development

An explanation and justification for the composition and presentation of the vaccine has been provided. Reasonable justification is given regarding the relevance of the chosen vaccine strain within the EU. The strain was isolated from a sheep in the Netherlands in September 2023 and is therefore clinically relevant to address the need for a vaccine against the currently emerging BTV 3 serovar, for which there is little to no cross-protection from vaccines against other BTV serovars.

The adjuvants, purified saponin and aluminium hydroxide, and other excipients; thiomersal, silicone antifoam and PBS used for the vaccine are the same as those already used for the multi-strain Syvazul BTV vaccine, which is authorised within the EU. Sufficient justification was provided for the use of the preservative, with satisfactory demonstration of efficacy of preservation provided.

The manufacturing of the active substance is briefly described and follows a similar format to that used for the multi-strain vaccine Syvazul BTV. The process has been specifically optimised for the BTV3 serovar. Furthermore, the inactivation kinetics and process validations are specific to Syvazul BTV 3 and described in part 2B and part 2D.

The manufacturing process of the finished product also follows a similar format to the multi-strain product, including all finished product testing with the exception of potency. The final potency test for the product is proposed as a serovar specific method, as for the BTV antigens used in the multi-strain

vaccine, however this method is still under development. As an interim potency test, RT-qPCR which detects the BTV- VP7 genome segment will be used, which is shown to correlate with the viral titre. This is acceptable, however a timeline for development of the potency method should be clarified. This is considered a specific obligation that the applicant will need to fulfil by January 2026.

Considering the exceptional circumstances, safety data from the existing Syvazul BTV multi-strain dossier is used, alongside additional safety parameters recorded from efficacy studies and suspected adverse reactions recorded after vaccination with Syvazul BTV 3 in the Netherlands, Belgium and Germany from April 2024. Efficacy is assessed in studies performed under laboratory-controlled conditions in the target species. As such, there is no list of batches that have been used in pivotal safety and efficacy trials in part 2A, which is acceptable.

Description of the manufacturing method

The process is considered to be a standard manufacturing process and is the same as that used for the authorised multi-strain Syvazul BTV vaccine. The manufacturing process consists of five main steps:

1. Production of the BHK-21 cell line

BHK-21 cells are cultured in culture flask or in bioreactor. The culture conditions are similar between the two environments. In both culture environments the passage number from the master seed must not exceed 20 passages, in accordance with Ph. Eur. 5.2.4.

2. Production of the inactivated BTV 3 antigen

Viral antigen is obtained by infection of the cell cultures until an adequate cytopathic effect (CPE) or cell viability rate are achieved.

The resultant viral antigen is harvested and filtered to remove cell debris, and samples collected for sterility and titration tests.

The viral suspension is inactivated by incubation with binary ethylenimine (BEI). The BEI is neutralised by adding an excess of sodium thiosulphate. The neutralised antigen is stored in sterile containers and samples collected for sterility, inactivation and residual sodium thiosulphate testing. The inactivated antigen can be stored at 5 ± 3 °C for up to 12 months. However, a post-authorisation measure is identified (to provide data in support of this storage period). This is considered a specific obligation that the applicant will need to fulfil by January 2027.

Inactivation kinetics and inactivation control tests have been performed demonstrating that four batches were inactivated within 67 % of the total inactivation time. The validation of the inactivation control tests is considered adequate and in line with the requirements of Ph. Eur. 0062.

3. Preparation of the vaccine

Sufficient information has been provided on the preparation of components, which include saponin solution and thiomersal solution, aluminium hydroxide gel, PBS and silicone antifoam as well as on the mixing operations.

4. Vaccine filling and packaging

The vaccine is sent to the dosing tank. The specified volume allowing for overages for each presentation is considered acceptable. Filling of the product is performed with shaking and the volume tested periodically.

5. Testing, storage and batch release

Testing of the finished product is performed by the quality control department. The finished product is stored at 5 ± 3 °C until confirmation of the result of the quality control testing. Any data outside the acceptance criteria will lead to rejection of the batch.

Major steps of the manufacturing process have been validated by three consecutive batches. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible and consistent manner. The in-process controls are adequate for this type of manufacturing process.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Certificates of analysis (CoA) from the supplier have been provided for all starting materials listed in the Pharmacopoeia, all conforming to Ph. Eur. specifications.

Bovine serum is used as a component in the culture media for the growth of BHK-21 cells. A certificate of analysis is provided for each supplier and country of origin, which describes the testing performed, including testing for a number of extraneous agents, sterility, mycoplasma and includes certificates of irradiation relevant for each CoA. Valid Certificate of Suitability to the European Pharmacopoeia (CEPs) have been provided for suppliers of bovine serum.

Gentamicin sulphate is used as a component of the culture media prior to infection, which is in compliance with Ph. Eur. 0062. This is considered acceptable.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

1. BTV-3/NET2023 strain of the bluetongue virus (serotype 3)

The virus strain BTV-3/NET2023 (serotype 3 of BTV) was isolated in September 2023 from the blood of a sheep in the Netherlands. The strain was isolated, passaged and then transferred to Laboratorios Syva.

The master seed virus (MSV) was prepared and tested for sterility in accordance with Ph. Eur. 2.6.1; for mycoplasma in accordance with Ph. Eur. 2.6.7; for identity; for viral titre and extraneous agents.

With regard to the testing for extraneous agents, a risk assessment is provided as well as a report describing the testing of all relevant ovine and bovine agents listed in Ph. Eur. 5.2.5. Considering the cell line is already in use for the manufacturing of authorised BTV vaccines this is adequate justification for the omission of testing for rodent (hamster) extraneous agents for this article 25 procedure. Considering the risk assessment provided, which includes bibliographic evidence of

inactivation of model viruses by BEI, as well as justification for not testing a number of agents, and the testing performed, the extraneous agent testing are considered acceptable.

A TSE risk assessment in accordance with '*Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products*' and Ph. Eur. 5.2.8 is provided. The BTV-3 isolate used in the production of the vaccine originated from an infected sheep from the Netherlands. Regarding the risk of the viral seed to be contaminated with TSE, the Netherlands belongs to the "negligible risk" countries, and therefore the risk of transmitting TSE is considered negligible.

The working seed virus (WSV) is prepared from the MSV in compliance with Ph. Eur. 0062. The WSV was tested for sterility in accordance with Ph. Eur. 2.6.1; absence of mycoplasma in accordance with Ph. Eur. 2.6.7 and viral titre. The MSV and WSV are stored at -80 °C.

2. BHK-21 cell line

The BHK-21 cell line master seed used for the production of the BTV 3 antigen is the same master cell seed used in the manufacture of the Syvazul multi-strain vaccine, therefore the details regarding origin, passage history and controls are the same as those found in the multi-strain dossier.

The BHK-21 cell line was established from hamster kidney cells. . The MCS is tested for general microscopy; sterility in accordance with Ph. Eur. 2.6.1; mycoplasma in accordance with Ph. Eur. 2.6.7; extraneous agents; identity; karyotype and absence of abnormal prion protein (PrP). The tests proposed are in accordance with Ph. Eur. 5.2.4. Testing for tumourigenicity is not performed, which is acceptable for an inactivated vaccine, considering no live cells will be present in the finished product.

With regard to extraneous agent testing, agents were tested based on volume 7B of EudraLex, section 7BIm10a, alongside a risk assessment for specific agents. The same cell line is used in the production of the BTV1, 4 and 8 serovars present in the Syvazul BTV multi-strain vaccine, therefore the omission of testing in accordance with Ph. Eur. 5.2.5 is considered acceptable.

A TSE risk assessment has been provided, which addresses the combined risk of the cell seed and products of animal origin in the cell culture media). In addition, the MCS is assessed for the expression of PrP, which would indicate the presence of TSE in the cell line. Considering the species of origin of the cells in not a TSE relevant species, the absence of PrP in the cells tested, the country of origin of the FBS and provision of valid CEP, the TSE risk can be considered as negligible.

3. Saponin

The saponin used is Quil-A, a purified extract of *Quillaia saponaria* tree bark. A CoA is provided from the supplier including a statement that no reagents of animal origin are used at any step of the production and therefore there is no associated TSE risk or risk of extraneous agent transmission. The manufacturer also confirms that no poyivinylpyrrolidona (PVP) or polyvinylpolypyrrolidona (PVPP) is used in the purification of saponin used in this vaccine, which considering the potential link between administration of vaccines containing povidone and incidence of anaphylaxis in cattle, is considered acceptable. The saponin solution is filtered prior to addition to the vaccine bulk, with satisfactory information on filter integrity provided.

4. Trypsin-EDTA solution

A CoA from the supplier is provided stating that the trypsin is irradiated and tested for PPV and PCV. A certificate of irradiation for the same trypsin-EDTA solution was provided in the multistrain dossier. The manufacturer also provides a TSE risk assessment stating that the risk of TSE is negligible.

5. Tryptose phosphate broth

A CoA for the tryptose phosphate broth is provided and it can be considered that the TSE risk for this product is negligible.

Starting materials of non-biological origin

The starting materials of non-biological origin not listed in a pharmacopoeia used in the manufacture of Syvazul BTV 3 are the same as those used in the multi-strain vaccine. CoA have been provided for all starting materials of non-biological origin confirming that the products are animal component free.

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

The media used in the manufacture of Syvazul BTV 3 are the same as those used in the multi-strain vaccine: BEI solution, cell line culture medium (MEM BHK-21) and phosphate buffered saline (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

During the manufacture of the antigen the following tests are carried out: appearance of the cell culture, passage number check, cell count, sterility, viral titration, inactivation controls, residual thiosulphate and pH. Test descriptions and the limits of acceptance were presented. The in-process tests are deemed to be sufficient to control all the critical steps in the manufacturing.

Titration of the virus is performed. No validation documents are provided, however the exact same method is used to determine viral titre of BTV 1, 4 and 8 antigens and was fully validated for each strain. Full validation is not required, as advised in the '*Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances*', the control tests must be demonstrated as fit for purpose. Considering the method is identical to the validated methods for BTV 1, 4 and 8, the risk of absence of validation data for BTV3 virus titration is considered very low and as such acceptable for this article 25 procedure.

The control of inactivation is determined in the viral antigen after inactivation. Satisfactory validation of the control of inactivation has been provided.

Determination of residual sodium thiosulphate is quantified in the viral antigen after inactivation in accordance with Ph. Eur. 0414. The presence of sodium thiosulphate must be detected to ensure complete neutralisation of BEI.

Control tests on the finished product

The description of the following methods used for the control of the finished product was provided: appearance, pH, volume, secondary packaging, identification of the active substance, batch titre or potency, identification and quantification of adjuvants (saponin and aluminium hydroxide), identification and quantification of preservative (thiomersal) and sterility.

Identification of the active substance is determined using specific reverse transcriptase PCR (RT-PCR) for detection of BTV 3 immediately prior to adding the fraction containing the adjuvant, as this

component interferes with the test. The RT-PCR was designed to detect a specific fragment of the Seg-2 gene encoding the VP-2 protein specific to BTV 3. The methods are adequately described; full validation was performed by the developers of the test. The applicant showed the suitability of the method and repeated the specificity testing for relevant viruses likely to be handled at the manufacturing facility (notably for BTV 1, 4, 8 and EHDV8).

A specific method for identification and quantification of the active substance in the final product, as potency test is still under development.

An interim potency method has been developed based on the quantification of the active substance by a BTV quantitative RT-PCR (RT-qPCR). Quantification is carried out during blending just prior to the addition of the fraction containing the adjuvant, as this component interferes with the test. The methods are adequately described, however, no validation document is provided. Considering that the application is under article 25, it is considered acceptable that the vendor calibration report is not provided in the dossier; at this point the method is considered to be fit-for-purpose. The acceptance criterion is included, however, it is unclear how this value relates to the clinical efficacy of the vaccine and therefore how it could be ensured that sub-potent batches could be adequately detected. Currently the potency is stated as the titre prior to inactivation and this is the most important quality attribute. While awaiting the development of the final potency test, the interim test can serve to confirm that sufficient quantity of BTV antigen is present in the vaccine. This can be temporarily accepted considering the application in exceptional circumstances but the submission of data relevant to the final potency test needs to be provided. This is considered a specific obligation that the applicant will need to fulfil by January 2026.

The quantity of saponin present in the finished product is determined and the test adequately described. The test is performed as validated (in accordance with VICH GL1 and GL2) for the Syvazul BTV multi-strain vaccine, which has the same adjuvant and excipient composition as Syvazul BTV 3. This is considered acceptable.

The quantity of aluminium in the vaccine is determined in accordance with Ph. Eur. 2.5.13. The methods, validation and acceptance criterion are the same as those for the Syvazul BTV multi-strain vaccine and therefore acceptable.

The quantity of thiomersal present in the finished product is determined in accordance with Ph. Eur. 2.2.23. The methods, validation and acceptance criterion are the same as those for the Syvazul BTV multi-strain vaccine and therefore acceptable.

Sterility in the finished product is determined in accordance with Ph. Eur. 2.6.1 '*Sterility*'.

Batch-to-batch consistency

The applicant presented finished product data for the manufacture of three consecutive finished product batches of the 200 ml presentation. The in-process tests are deemed to be sufficient to control all the critical steps in the manufacturing process.

The results of finished product testing for the three vaccine batches were highly consistent, supportive of a well-controlled manufacturing process.

Data were only provided from the 200 ml presentation, with no batches of the 80 ml presentation. The packaging is the same material between the 80 ml and 200 ml presentations and there are no concerns with the consistency data presented for any batch of the 200 ml presentation. This approach is acceptable in this case and in line with the '*Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances*'

(EMA/CVMP/IWP/251947/2021), which requires data from at least two pilot or R&D batches, without making reference to the different presentations.

Stability

Stability data for Syvazul BTV 3 are not available. However, in accordance with the '*Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances*' (EMA/CVMP/IWP/251947/2021), stability data of a vaccine containing other serotypes but having the same composition in adjuvants and excipients may be used to define the shelf life. Therefore, the shelf life is defined based on data from the Syvazul BTV multi-strain vaccine.

- Active substance:12 months at 5 ± 3 °C
- Vaccine: 2 years at 5 ± 3 °C
- Vaccine after first opening: 10 hours at room temperature

This approach is considered acceptable as the composition of the adjuvants and excipients are identical between Syvazul BTV multi-strain and Syvazul BTV 3, with the exception of the serovar of the active substance, and is also in line with the advice from Guideline EMA/CVMP/IWP/251947/2021. However, a recommendation as a post-authorisation measure is requested to provide data to confirm the proposed shelf life and the recommended storage conditions for the active substance and the finished product. This is considered a specific obligation that the applicant will need to fulfil by January 2027.

New active substance (NAS) status

The applicant requested the active substance Bluetongue virus, serotype 3, BTV-3/NET2023, inactivated contained in Syvazul BTV 3 to be considered a new active substance as it is novel and not hitherto authorised in a veterinary medicinal product in the European Union.

Based on the review of the data provided, the CVMP considered that the active substance inactivated bluetongue virus, serotype 3, strain BTV-3/NET2023 contained in the veterinary medicinal product Syvazul BTV 3 is not to be qualified as a new active substance considering that another vaccine which contains inactivated bluetongue virus, serotype 3 was granted a marketing authorisation in the EU in October 2024.

Overall conclusions on quality

The quality part of the dossier complies with the Annex II to Regulation (EU) 2019/6 taking into consideration this application has been made under Article 25 '*Applications in exceptional circumstances'* with reference to the 'Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances' (EMA/CVMP/IWP/251947/2021). General, and where relevant, specific Ph. Eur. monographs have been followed and the data are generally adequate in support of a consistent and well controlled manufacturing process.

Considering the application is under exceptional circumstances, several data gaps were identified in the dossier provided. The product development and description of manufacturing sections provided are considered adequate; however, limited information and validation are provided for production seeds derived from reactors. With regard to the starting materials, there are some gaps in the extraneous agent testing including for bovine serum and the BHK-21 cell line. This is considered justified as the inactivation of the vaccine with BEI could be considered sufficient to inactivate viral

agents, furthermore the BHK-21 cell line is currently used in the production of the multi-strain Syvazul BTV vaccine. No validation is provided for the in-process titration of the virus, which is considered acceptable as validation is performed for the other BTV antigens included in the Syvazul multi-strain BTV vaccine. With regard to the potency testing, an interim test is proposed determining the number of copies of generic BTV by RT-qPCR. Validation is not provided and the method determining the acceptance criteria is not clear. The method proposed is not considered ideal, however, considering that the primary determination of potency is the pre-inactivation titre, upon which the qualitative composition of the active substance is based, this can be considered acceptable. A specific obligation has been added to develop the final potency test, including a timeline for completion. The shelf-life is determined based on the shelf-life for the multi-strain Syvazul BTV vaccine. This is considered acceptable, however, a post-authorisation recommendation is included to provide specific data from the Syvazul BTV 3 vaccine confirming the proposed shelf-life and storage conditions.

The composition of the product is described in sufficient detail. The development of the product has been adequately described and justified, with the gaps highlighted above. All excipients are well known pharmaceutical ingredients and there are no novel excipients used in the finished product formulation. Furthermore, the composition of the vaccine in terms of the adjuvant and excipients is the same as that of the already centrally authorised Syvazul BTV multi-strain vaccine.

The manufacturing process consists of two main steps for the antigen, followed by blending of the finished product and filling into final containers. The manufacturing process has generally been described in adequate detail.

Starting materials have been listed and shown to comply with pharmacopoeial or in-house requirements. The extraneous agents risk assessment for the MSV is sufficient. Description of the media and working solutions is adequate.

Control tests performed during the manufacturing process have generally been adequately described. Considering this application is made under Article 25, full validation does not need to be provided, test methods were shown to be fit for purpose.

Finished product control tests have generally been adequately described and testing of the adjuvants and excipients have been appropriately validated. The potency test is proposed, however, this is under development and therefore an interim method detecting the VP7 portion of the BTV gene by RT-qPCR is proposed. Since this interim test is not very accurate the applicant is requested to indicate a timeframe for the development of the final test, this is requested as a specific obligation. The identity test was sufficiently validated and shown to be suitable.

Consistency of manufacture has been adequately supported by data from three consecutive batches.

No stability data are provided, with the shelf life extrapolated from the Syvazul BTV multi-strain vaccine. This is considered acceptable in line with the '*Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances*' (EMA/CVMP/IWP/251947/2021); however, a post-authorisation specific obligation has been added for the applicant to provide data to confirm the proposed shelf life.

Based on the review of the data on quality, the manufacture and control of Syvazul BTV 3 are considered acceptable in the framework of exceptional circumstances.

Part 3 – Safety documentation (safety and residues tests)

General requirements

The current application for a marketing authorisation for the product 'Syvazul BTV 3' has been submitted in accordance with Article 25 of Regulation (EU) 2019/6, application in exceptional circumstances.

In support of the safety of the product for the originally proposed target animal species sheep and cattle, users, consumers and the environment, the applicant has referred to those data that were submitted for the authorised multi-strain vaccine, 'Syvazul BTV suspension for injection for sheep and cattle' which contains a maximum of 2 of the following inactivated bluetongue virus serotypes: BTV serotype 1, 4, 8. A similar regulatory approach to that taken for multi-strain dossiers can be taken for this application, noting that the VMP contains a single strain of BTV (serotype 3, BTV-3/NET2023 (inactivated)) and therefore a lower antigenic burden than the authorised multi-strain vaccine which can contain up to two BTV strains. Moreover, the same adjuvants and excipients in the same quantities are included in the authorised multi-strain vaccine. Furthermore, the product was initially proposed for use in the same target species according to the same vaccination schemes as the authorised multi-strain vaccine.

As the data concerned have been accepted by CVMP, reference is made to the summary of the CVMP's assessment as contained in the EPAR for 'Syvazul BTV suspension for injection for sheep and cattle' (EMA/802733/2018).

In addition to those data already accepted for the multi-strain dossier, the applicant has investigated safety of the vaccine Syvazul BTV 3 in the context of two pre-clinical studies performed in sheep that were primarily designed to investigate efficacy. The applicant has also provided pharmacovigilance data gathered since authorisation of 'Syvazul BTV 3' for emergency use in the face of a BTV-3 outbreak in a small number of MS.

Safety documentation

The applicant has investigated the safety of the Syvazul BTV 3 vaccine in two pre-clinical laboratory studies in sheep, which were, however, designed with the primary goal of demonstrating efficacy. These studies are summarised below.

Study ID	Study Title	Animals	Treatment groups
01	Efficacy of two inactivated BTV-3 vaccine candidates against an experimental BTV-3 challenge in sheep	Non-pregnant ewes 11 – 12 months old	T01 (n=8): placebo (saline) T02 (n = 8): BTV-3 vaccine A T03 (n = 8): BTV-3 vaccine B
02	Serological efficacy study following administration of two BTV-3 vaccine candidates to lambs	Lambs, (15 ♂, 15 ♀) 4 months old at study day 0	Group 1 (n = 12): BTV-3 vaccine 2 Group 2 (n = 12): BTV-3 vaccine 1 Group 3 (n= 6): placebo (PBS)

Pre-clinical studies

Safety of the administration of one dose and repeated doses

The conclusions reached by CVMP in respect of the safety of administration of a single and repeated dose of the authorised multi-strain vaccine 'Syvazul BTV-1+8' are noted and are considered relevant and applicable to the current MAA for 'Syvazul BTV 3.' Namely, these conclusions are:

Sheep: '…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 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.'

In the context of the current application, the applicant has also evaluated safety parameters in two pre-clinical laboratory studies. Although the studies in question were not designed primarily as safety studies, this approach is foreseen in the relevant Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances (EMA/CVMP/IWP/251947/2021): *'The safety and efficacy studies may be combined in the same pre-clinical (laboratory) study'*; and is considered acceptable. These studies were not GLP-compliant, however, in accordance with the relevant guidance non-compliance of these studies with GLP can be accepted.

All batches used were manufactured as described in Part 2 of the dossier and vaccine batches contained the same concentration of inactivated virus, that is $10^{7.2}$ $CCID_{50}$/mL.

In study 01, female sheep aged 11-12 months of age were used, although the candidate vaccine is proposed for use in sheep from 3 months of age. Animals were randomly assigned to one of three groups, each with 8 animals. Group T01 was a placebo control group, and these animals received 2 ml saline subcutaneously. Animals in groups T02 and T03 received the candidate vaccine, which was administered in accordance with the proposed SPC, that is, 2 ml administered subcutaneously on one occasion. Animals were monitored for general clinical signs, rectal temperature and local reactions at the site of injection (both *in vivo* and at necropsy at the conclusion of the study).
No clinical signs attributable to vaccine administration were reported. In respect of rectal temperature, no animals presented with temperatures > 40 ºC (which was the cut-off value used for pyrexia in this study) following vaccination, and the maximum increases in temperature (0.9 ºC and 0.8 ºC in groups T02 and T03 respectively) as compared to an average of 3 basal temperature values, were observed on study day 1, at 24 hours post-vaccination.

No local reactions at the injection site were observed in any animal during the *in vivo* phase of this study. At necropsy on study day 42 however, firm granulomatous nodules (ranging from 0.5 – 2 cm in diameter) at the injection site were observed in n=6 animals in T02 (vaccine A) and in n=7 animals in T03 (vaccine B). No nodules were observed in animals in T01 (placebo control). The possibility that residual nodules can persist at the injection site for at least 70 days post-vaccination is captured in section 3.6 of the proposed SPC.

In the proposed SPC, information in section 3.6 is the same as that in the authorised SPC for the multi-strain vaccine. It is noted that hyperthermia and injection site reactions are included in section 3.6 as very common adverse events. Notwithstanding the fact that the safety data from study 01 were not generated in animals of the minimum age, no further update to the proposed SPC is necessary based on the results of this study.

In study 02, lambs that were 4 months old on the day of first vaccination were used which can be accepted as being suitably representative of animals of the minimum age. Animals were randomly assigned to one of 3 groups: Group 1 (n = 12) and Group 2 (n = 12) received the candidate vaccine, and Group 3 (n = 6) received PBS and acted as a placebo control group.

On study day 0 animals in Group 1 and Group 2 received a dose of the candidate IVMP in accordance with the proposed SPC, that is 2 ml administered subcutaneously (in the right axilla), and animals in Group 3 were administered 2 ml PBS subcutaneously, also in the right axilla. On study Day 28, animals were administered a second dose of vaccine / PBS, this time in the left axilla. Administration of a second dose 4 weeks after administration of the first is not consistent with the primary vaccination schedule as currently proposed in section 3.9 of the SPC.

Animals were observed for general clinical signs, rectal temperature and local injection site reactions, and a suitable schedule for observation of these safety parameters was used. No clinical signs that are likely to be related to vaccine administration were observed following the first and second vaccination. In respect of rectal temperature, the maximum increase in rectal temperature (0.47 ºC and 0.39 ºC in groups 1 and 2 respectively) as compared to an average of 3 basal temperature values, was observed at 24 h post first vaccination and a similar pattern was observed following the second vaccination, that is that the maximum increase in rectal temperature (1.49 ºC and 0.9 ºC in groups 1 and 2 respectively) was observed at 24 h post second vaccination. No further update to section 3.6 of the proposed SPC is considered necessary based on these data.

In respect of injection site reactions, no control animals presented with reactions, whereas most animals developed a local reaction following the first vaccination, and all animals had a local reaction following the second vaccination. In general, the reactions presented as swelling, with or without erythema and pain, which developed into firm / indurated nodules over time. Following the first vaccination, no animal was reported to have pain, and redness (observed in 3 animals each in group 1 and 2) resolved in most animals by 3 days post vaccination. Swelling was observed in the majority of animals following vaccination (10/12 in each treatment group) and became indurated (nodules) between days 6 – 10. By day 40 nodules were still palpable in 3/12 lambs in group 1 and 3/12 lambs in group 2. These remaining nodules were 1.1 – 1.8 cm in diameter. Noting that these data are representative of use of the vaccine in accordance with the proposed SPC in animals of approximately the minimum age, it is considered that the injection site reactions observed are suitably captured by the description of injection site reactions in section 3.6 of the proposed SPC.

Following second vaccination, all animals developed swelling at the injection site, one animal in each treatment group had pain on palpation and redness was observed in 6/12 and 3/12 animals in groups 1 and 2 respectively. Similar to the pattern observed following the first vaccination, the swelling observed developed into nodules in all animals, and by 12 days post vaccination these nodules were 2.1 – 4 cm in diameter. It is noted that the size of both the swelling (area > 12 cm^2) and nodules (area > 8 cm^2) that developed following the second vaccination were greater than after the first vaccination.

It is accepted that based on this study, if the candidate vaccine is used in accordance with the proposed SPC in the target animal species sheep, information in section 3.6 of the SPC concerning these adverse events specifically, can be considered adequate.

In respect of the originally proposed target animal species cattle, no further safety data generated using the candidate vaccine have been provided by the applicant. Information concerning adverse events in cattle in the proposed SPC is consistent with that proposed for the authorised multi-strain vaccine.

It is concluded that safety of administration of a single and repeated dose of the candidate IVMP 'Syvazul BTV 3' has been suitably investigated based on the data presented.

Safety of one administration of an overdose

The conclusions reached by CVMP in respect of the safety of administration of an overdose (in sheep only) of the authorised multi-strain vaccine 'Syvazul BTV-1+8' are noted. Namely, these conclusions are:

Sheep: '…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.'

The following information has been proposed for inclusion in section 3.10 of the SPC for the candidate IVMP and is considered acceptable: The safety of an overdose has not been established.

Examination of reproductive performance

The conclusions reached by CVMP in respect of examination of the effect on reproductive performance of the authorised multi-strain vaccine 'Syvazul BTV-1+8' are noted and are considered relevant and applicable to the current MAA for 'Syvazul BTV 3.' Namely, these conclusions are:

Sheep: '…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.'

The following conclusions concerning reproductive performance in sheep were also reached based on the field studies submitted: '…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' and '…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.'

It is concluded by CVMP that safety of administration of the candidate IVMP 'Syvazul BTV 3' to pregnant and lactating animals may be considered to have been suitably investigated based on the data presented. Section 3.7 of the proposed SPC that concerns use of the candidate IVMP in pregnant and lactating animals contains the same information as that included in section 3.7 of the authorised SPC for the multi-strain vaccine, namely that the vaccine can be used during pregnancy and lactation, which is considered acceptable. It is also stated in section 3.7 that the safety of the candidate vaccine has not been established in breeding male animals, which is consistent with the authorised multi-strain vaccine, and is also considered acceptable.

Examination of immunological functions

The conclusion reached by CVMP in respect of examination of immunological functions of the authorised multi-strain vaccine 'Syvazul BTV-1+8' (namely that no adverse effects on immunological function are anticipated) is noted and is also considered applicable to the current application for the candidate VMP which is also an inactivated vaccine containing (the same) compounds that have no known adverse effect on immunological function.

User safety

In respect of user safety, it was concluded by CVMP that for the multi-strain vaccine '…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.'

As the candidate vaccine also contains inactivated bluetongue virus (BTV-3) and the same excipients (inclusive of the adjuvants aluminium hydroxide and purified saponin (Quil-A) from Quillaja Saponaria) as the authorised multi-strain vaccine, and is currently proposed for use via the same route of administration and according to the same vaccination schedule, it is concluded that use of the candidate vaccine will not pose a different risk to the user than that posed by the authorised vaccine. General user safety warnings are included in the SPC and are considered acceptable.

Study of residues

The conclusions reached by CVMP in respect of the risk posed to the consumer by the authorised multi-strain vaccine 'Syvazul BTV-1+8' are that 'Syvazul BTV' is expected to pose a negligible risk for the consumer; the withdrawal period is set at zero days.' As the active substance in the candidate vaccine is of biological origin intended to produce active immunity and as such, is not within scope of Regulation (EC) 470/2009, use of a different inactivated BTV serotype in the candidate IVMP will not increase the potential for the candidate IVMP to pose a risk to the consumer as compared to the authorised multi-strain vaccine. Furthermore, the excipients and adjuvants are the same as in the multi-strain vaccine. The same conclusion concerning consumer safety can be reached for the candidate IVMP and the proposed withdrawal period of zero days is considered acceptable.

Interactions

In respect of interactions with other veterinary immunological products, these have not been investigated for either the authorised multi-strain vaccine, or the candidate vaccine. As such, the proposal of the applicant to include standard text reflecting this information in section 3.8 of the SPC is considered acceptable.

Clinical studies

Based on the clinical trials provided in support of the multi-strain dossier, the following conclusions concerning target animal safety were reached by CVMP:

Sheep

'…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 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 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.'

A summary of pharmacovigilance data concerning the candidate IVMP which was authorised for emergency use in a small number of MS in the face of an ongoing BTV-3 outbreak has been provided. These data were gathered between April and July 2024. Adverse events were reported and comprised both safety events and lack of expected efficacy (LEE) events. For the purposes of the assessment of target animal safety, safety reports will be considered under Part 3, and reported LEE will be taken into consideration under Part 4, Efficacy.

Concerning sheep, the most commonly reported VeDDRA preferred terms (PT) were death, anorexia, lethargy and ruminant stomach disorders. These adverse events are calculated as having the frequency 'very rare.'
In the proposed SPC, anorexia and lethargy are included as 'rare' adverse events, whereas death and rumen atony, bloated are included as 'very rare.'
Based on a comparison of the pharmacovigilance data and section 3.6 of the proposed SPC, it is concluded that the adverse event profile of the candidate IVMP as used in the field in the target animal species sheep is suitably reflected in the proposed SPC.

Concerning cattle, the most commonly reported PT terms were: lactation change, milk production decrease, hypersalivation, high somatic cell count, diarrhoea, anorexia, abortion lethargy, and premature parturition. All PTs were reported as 'very rare' with the exception of milk production decrease and anorexia which were 'rare'.

It is concluded that the pharmacovigilance data provide reasonable reassurance that when used in accordance with the SPC, the safety profile of the candidate VMP is similar to that of the authorised multi-strain VMP.

Environmental risk assessment

The conclusions reached by CVMP in respect of the risk posed to the environment by the authorised multi-strain vaccine 'Syvazul BTV-1+8' are as follows: ''Syvazul BTV' is expected to pose a negligible risk for the environment when used according to the SPC.' Use of a different inactivated BTV serotype in the candidate IVMP will not increase the potential for the candidate IVMP to pose a risk to the environment as compared to the authorised multi-strain vaccine, and as such, the same conclusion can be reached for the candidate IVMP.

Overall conclusions on the safety documentation

In support of safety for the candidate IVMP 'Syvazul BTV 3' the applicant has referred to those safety data accepted by CVMP in the context of the application for a marketing authorisation for the authorised multistrain vaccine 'Syvazul BTV suspension for injections for sheep and cattle' (which contains a maximum of 2 of the following inactivated bluetongue virus serotypes: 1, 4, 8). In the context of this application, that is, in accordance with Article 25 of Regulation (EU) 2019/6, the approach of the applicant (i.e., reference to safety data generated using the multistrain vaccine rather than 'Syvazul BTV 3' specifically) can be considered in accordance with the Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances (EMA/CVMP/IWP/251947/2021), and accepted. The applicant has also provided two pre-clinical studies in which safety of a single and repeated dose via the recommended route of administration in the target animal species sheep (but not cattle), was investigated. Batches used in these studies were manufactured in accordance with Part 2 of this dossier.

On the basis of the results of these pre-clinical studies and taking into account those safety data that have been accepted by CVMP for the authorised multi-strain vaccine, it is concluded that the safety of the originally proposed target animals when the product is administered according to the recommended schedule and via the recommended route is acceptable. However, before authorisation of Syvazul BTV 3, the applicant withdrew cattle as a target species. Please refer to Part 4 (Efficacy) for more information.

The following information has been proposed for inclusion in section 3.10 of the SPC for the candidate IVMP and is considered acceptable: The safety of an overdose has not been established.

Section 3.7 of the proposed SPC that concerns use of the candidate IVMP in pregnant and lactating animals states that the vaccine can be used in during pregnancy and lactation. It is also stated in section 3.7 that the safety of the candidate vaccine has not been established in breeding male animals.

No adverse effects on immunological function are anticipated.

'Syvazul BTV 3' does not pose any specific risk to the user when used as recommended, and consistent with the SPC of the authorised multistrain vaccine, general user safety warnings are included in the SPC.

Interactions of 'Syvazul BTV 3' with other veterinary medicinal products has not been investigated, which is reflected in the SPC.

A summary of pharmacovigilance data generated from use of the candidate IVMP in a small number of MS (for emergency use in the face of an ongoing BTV-3 outbreak) was also submitted in the context of the current application.
Based on a comparison of the pharmacovigilance data and section 3.6 of the proposed SPC, it is concluded that the adverse event profile of the candidate IVMP as used in the field in the target animal species sheep is suitably reflected in the proposed SPC.

'Syvazul BTV 3' is expected to pose a negligible risk for the environment when used according to the SPC.

'Syvazul BTV 3' is expected to pose a negligible risk for the consumer; the withdrawal period is set at zero days.

It is concluded by CVMP that safety of the candidate IVMP has been suitably investigated. The candidate IVMP is accepted as being safe for the target animal species sheep, the user, the consumer and the environment when used in accordance with the proposed SPC.

Part 4 – Efficacy documentation (pre-clinical studies and clinical trials)

General requirements

Syvazul BTV 3 is an inactivated vaccine proposed for use in sheep and cattle which has been developed under emergency circumstances due to the highly virulent BTV serotype 3 outbreaks in sheep reported from September 2023 in Europe. For this rapid development process, the applicant has used the existing vaccine development and production knowledge and data from its portfolio of BTV vaccines authorised under the Syvazul BTV multi-strain dossier (EU/2/18/231/001-012).

The vaccine strain included in Syvazul BTV 3 is BTV-3/NET2023 (serotype BTV-3) was isolated from sheep during the recent outbreak in the Netherlands in September 2023. This outbreak marked the beginning of a fast-expanding epidemic that has subsequently spread to Belgium, Germany and Great Britain. The choice of the vaccine strain is acceptable and is directly relevant to the current epidemic in the EU caused by serotype 3 of BTV.

The current application is submitted in accordance with Article 25 of Regulation (EU) 2019/6, i.e., an application under exceptional circumstances, which states "By way of derogation from point (b) of Article 8(1), in exceptional circumstances related to animal or public health, an applicant may submit an application which does not meet all requirements of that point, for which the benefit of the immediate availability on the market of the veterinary medicinal product concerned to the animal or public health

outweighs the risk inherent in the fact that certain quality, safety or efficacy documentation has not been provided. In such a case, the applicant shall be required to demonstrate that for objective and verifiable reasons certain quality, safety or efficacy documentation required in accordance with Annex II cannot be provided."

In sheep, the vaccine is (initially) proposed for the active immunisation from 3 months of age to reduce viraemia, to prevent mortality and reduce clinical signs and lesions caused by bluetongue virus serotype 3. The claimed onset of immunity is 28 days after completion of the primary vaccination scheme. The duration of immunity has not been established. The primary vaccination scheme proposed in sheep consists of a single 2 ml dose, administered subcutaneously. A revaccination schedule of a 2 ml dose after 12 months is recommended.

In cattle, the vaccine is proposed for the active immunisation of cattle against bluetongue virus serotype 3 and is proposed for use from 2 months of age in naïve animals or from 3 months of age in calves born to immune cattle. The onset of immunity and duration of immunity have not been established. The primary vaccination scheme in cattle consists of two doses of 4 ml dose, separated by an interval of 3 weeks, administered intramuscularly. A revaccination schedule of a 4 ml dose after 12 months is proposed.

Information is included in the SPC to indicate the absence of data on the use of the vaccine in sheep with maternally-derived antibodies.

The minimum amount of antigen included in the vaccine is $10^{6.9}$ $CCID_{50}$ per ml, based on pre-inactivation titres, and was selected according to previous experience with the BTV multi-strain vaccine, using an antigen content or 'payload' which is considered to be well in excess of the minimum protective dose confirmed for the three strains of BTV included in the BTV multi-strain dossier. The vaccine is adjuvanted with aluminium hydroxide and saponin. The amount of antigen included in the vaccine for the batches used in the pre-clinical studies was higher than the minimum concentration selected; whilst this aspect is not in accordance with requirements for the evaluation of immunogenicity in line with Ph. Eur. 5.2.7 ("Evaluation of efficacy of veterinary vaccines and immunosera"), the use of a non-minimum antigen content batch is an acceptable derogation from Annex II requirements for efficacy studies, in accordance with the Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances (EMA/CVMP/IWP/251947/2021). This is an identified and accepted data gap for the efficacy data submitted in this Article 25 application.

In order to evaluate the efficacy of Syvazul BTV 3, and to rapidly facilitate the availability of the vaccine, the applicant has taken into account the recommendations of the Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances (EMA/CVMP/IWP/251947/2021). In accordance with this guideline, efficacy claims are demonstrated under laboratory conditions and no clinical field studies are required. Two pre-clinical studies consisting of vaccination and challenge were performed under laboratory conditions to demonstrate efficacy of Syvazul BTV 3 in sheep, while one pre-clinical study consisting of vaccination and measurement of serological response in calves was performed under laboratory conditions to support the use of the vaccine in cattle. Although no clinical field studies were conducted using Syvazul BTV 3, it is noted that data are available following use of this vaccine in the field in a number of EU member states following use in accordance with Article 110(2) of EU Regulation 2019/6.

Challenge model

The challenge model used was established by the Dutch National Veterinary Reference Laboratory for BTV; Wageningen Bioveterinary Research (WBVR). The challenge model consisted of two groups (n=6) of sheep inoculated by the subcutaneous route with two different concentrations of live virus divided over

two volumes of 2 ml given on the right and left side of each animal (virus concentration not stated, however it is assumed that the challenge dose administered informed the challenge study in sheep). Follow-up over 3 weeks consisted of scoring of clinical signs (depression, appetite, mucosal lesions, nasal discharge, dyspnoea, salivation, oedema and lameness), viraemia and development of antibodies. For animals in both groups, viraemia developed from day 3 post-challenge, fever developed from day 4 and severe clinical symptoms developed from day 7 onwards. From day 9 to 12 post-challenge, all but one infected animal was euthanised due to reaching of predefined humane endpoint criteria. This severe challenge model was used in sheep for evaluation of the onset of immunity. It is noted that detailed information regarding development of the challenge model is not provided. However, it would appear from the summary as presented in the dossier that the BTV 3 challenge model was capable of establishing a severe infection. Furthermore, information on the challenge dose and administration is provided in the two challenge studies conducted in sheep and is considered sufficient. The route of administration (subcutaneous) of the challenge dose is considered to mimic the natural infection route insofar as possible (biting midges of the *Culicoides* species).

The BTV-3 challenge strain used for the laboratory efficacy studies was homologous to the vaccine strain; BTV-3/NET2023 strain and was derived from a blood sample taken from a diseased sheep. The use of a homologous challenge strain, noting the Ph. Eur. 5.2.7 requirements to conduct challenges with heterologous strain, is addressed by the applicant. Published data on different phylogenetic analyses performed for other BTV serotypes showed a percentage of nucleotide identity of 90 % or higher in most of the cases between different viruses within a cluster. This indicates few points of mutations are acquired over a relatively long period of time with little or no mixing or exchange between them (Sushila Maan et al., 2008; Cêtre-Sossah et al., 2011). This behaviour was similar across BTV serotypes which indicates a pattern of few genome mutations among BTV-3 isolates from different locations in Europe. In addition, the emergency circumstances which require a rapid vaccine development process did not allow time nor a scientific rationale for a search for more heterologous field strains and the evaluation of these in animal models. It is noted by CVMP that it is stated in Ph. Eur. 5.2.7 that unless otherwise justified, challenge is carried out using a strain different from the one used in the production of the vaccine. In justifying this deviation from requirements, the most pertinent point was the need to expedite the development of a challenge model and insufficient time to source and determine the suitability of heterologous BTV-3 strains. Therefore, whilst this is an identified data gap for the efficacy data submitted in this Article 25 application, it can be considered that adequate justification is provided in respect of the use of a homologous challenge strain for challenge; concerning the current epidemiological situation in the EU, it is acknowledged that protection against BTV-3/NET2023 strain is highly relevant.

Efficacy parameters and tests

The efficacy parameters as chosen by the applicant, investigated in the efficacy studies, are clinical signs and lesions, pyrexia, viraemia, the level of antibodies against VP7 of BTV and BTV 3-specific virus neutralising antibody titres.

For 21 days post-challenge, sheep were observed for clinical signs related to the BTV infection at least once daily. During the period with expected clinical symptoms (day 5 to 14 post challenge), monitoring was performed twice daily. Clinical observations were conducted according to a scoring system which included monitoring of the following parameters; depression, appetite during feeding, nasal discharge, mucocutaneous lesions in the mouth, salivation, oedema of head and throat, dyspnoea, cough, locomotion abnormalities (posture and gait, lameness) and leg abnormalities (swelling/redness, overfilled joint, warm hoof), with scores of 0, 1, 2, and 3 indicating no signs, mild, moderate or severe signs, respectively. A daily composite clinical score was determined per treatment group by calculating the daily sum score combined for all clinical signs divided by the number of sheep present in the group. The scoring

system used for evaluation of clinical signs is considered to be appropriate for the evaluation of clinical signs of disease relating to BTV. Post-mortem was conducted to evaluate gross and histopathological lesions.

For viraemia, the presence and quantity of virus in blood was investigated by detection of the viral genome using a reverse transcriptase polymerase chain reactions (RT-qPCR) assay. Testing for viraemia was conducted at Wageningen Bioveterinary Research, the Netherlands' national reference laboratory for animal disease. As such, no validation of the method to evaluate viraemia is provided or considered necessary.

Commercially available ELISA tests were used to measure BTV antibodies in serum samples which detect antibodies against the VP7 nucleoprotein of the bluetongue virus, common to all the serotypes, and is specified for use in multiple species including sheep and cattle. Tests were performed at WBVR for the studies conducted in sheep, performed according to the manufacturer's specifications and instructions. No internal validation reports of the commercial ELISAs used in the sheep and cattle studies are deemed necessary.

The virus neutralisation (VN) test was used for the detection of specific BTV-3 neutralising antibodies in serum samples. For the sheep studies the test was performed at WBVR while for cattle it was performed at Syva. Serum samples were serially diluted on duplicate 96-well plates and incubated with BTV-3 (isolate homologue to the BTV-3 isolate used for vaccine production). Then a cell suspension of BSR cells (clone of BHK cell line) was added and the plates were further incubated. At the end of the incubation period, plates were washed and read by microscopic evaluation to determine the presence of cytopathogenic effect (CPE). Mean titres of BTV3 neutralising antibodies were expressed for each group as the geometrical mean titre (GMT) of the positive animals per timepoint. For calculations a Log2 transformation of the titres was previously performed. For graphical representation an arbitrary value of 1 was given to the GMT at the timepoints in which there were no positive animal in the group. The validation of the VN test for the detection of seroneutralising antibodies against BTV-3 has not been provided. However, at WBVR the method is performed under a quality system, includes adequate controls, is well-established and can be considered to be fit for purpose. At laboratorios Syva, virus neutralisation tests for BTV 1, 4 and 8 are performed according to an identical method and have been validated in the frame of the authorisation of the multistrain BTV vaccine. The method is well-established and includes appropriate controls, it is considered fit for purpose.

The parameters chosen are considered appropriate for evaluating the efficacy of the product.

Efficacy documentation

Three pre-clinical studies were conducted to investigate the efficacy of the product. Laboratory studies were well documented and carried out in the target species sheep, evaluating the onset of immunity by challenge after a single dose (proposed vaccination schedule) in sheep older than the minimum age recommended for vaccination and after a two-dose basic vaccination schedule (not the proposed vaccination schedule) in sheep of the minimum age recommended for vaccination. In both studies, the vaccine was administered by the recommended route. To demonstrate efficacy in the target species cattle, a laboratory study was carried out in calves of the minimum age recommended for vaccination by the recommended administration route, evaluating the onset of immunity by serology after a two-dose basic vaccination schedule (proposed vaccination schedule). The duration of immunity has not been investigated in either of the target species.

The pre-clinical studies were well documented and carried out using R&D / pilot batches or industrial batches of vaccine.

Study reference	Study title	Batch used
Sheep studies:		
01	Efficacy of two inactivated BTV-3 vaccine candidates against an experimental BTV-3 challenge in sheep – OOI after single dose	"Vaccine A"* $10^{7.2}$ $TCID_{50}$/ml "Vaccine B"** $10^{7.2}$ $TCID_{50}$/ml
02 and 03	Serological efficacy study following administration of two BTV-3 vaccine candidates to lambs (vaccination phase) and Efficacy of a prime boost administered BTV-3 vaccine against experimental BTV infection in sheep (challenge phase) - OOI after two doses	"Vaccine 1"* $10^{7.2}$ $TCID_{50}$/ml "Vaccine 2"*** $10^{7.2}$ $TCID_{50}$/ml
Cattle study		
04	Serum antibody efficacy study following the administration of two BTV-3 vaccine candidates to cattle – OOI after two doses	"Vaccine 1" $10^{6.9}$ $TCID_{50}$/ml "Vaccine 2" $10^{7.2}$ $TCID_{50}$/ml

*Syvazul BTV 3, virus concentration before inactivation $10^{7.2}$ CCID50/ml, used for manufacture of final lot vaccine batch Vaccine 1/Vaccine A. ("Vaccine A" used in study 01 and "Vaccine 1" in study 02 and 03). Study batches are R&D batches of vaccine, manufactured with an industrial batch of antigen.

**Syvazul BTV 3, virus concentration before inactivation $10^{7.2}$ CCID50/ml, used for manufacture of final lot vaccine batch Vaccine B. ("Vaccine B" used in study 01). Study batch is an R&D batch of vaccine, manufactured with an industrial batch of antigen.

*** Syvazul BTV 3, virus concentration before inactivation $10^{7.2}$ CCID50/ml, used for manufacture of final lot vaccine batch Vaccine 2, ("Vaccine 2" used in study 02 and 03). Study batch is an R&D batch of vaccine, manufactured with an industrial batch of antigen.

Syvazul BTV 3, virus concentration before inactivation $10^{6.9}$ CCID50/ml, used for manufacture of final lot vaccine. ("Vaccine 1" used in study 04). Industrial batch of vaccine, manufactured with an industrial batch of antigen.

Syvazul BTV 3, virus concentration before inactivation $10^{6.9}$ CCID50/ml, used for manufacture of final lot vaccine batch Vaccine 2. ("Vaccine 2" used in study 04). R&D batch of vaccine, manufactured with an industrial batch of antigen.

Syvazul BTV 3 uses a combination of aluminium hydroxide and saponin as adjuvants, this is the same combination as used in Syvazul BTV. The saponin included in Syvazul BTV 3 is the purified saponin recently approved by EMA for the vaccines in the BTV multi-strain dossier and does not contain any traces of povidone.

According to available guidance for Art. 25 applications for IVMPs, the CVMP notes that efficacy should be demonstrated in laboratory conditions by a challenge model in all recommended target species for vaccination unless scientific/literature data can be provided demonstrating that extrapolation from one species to another species is possible. Challenge data are provided in sheep, but no challenge data are available in cattle.

If an indicator of protection is used, the challenge may be omitted. For an indicator to be acceptable as a correlate of IVMP efficacy, it shall be demonstrated that a sufficient correlation exists between the indicator measured and the claimed protection in the target species. The applicant has used serology as an indicator of protection / response to immunisation in cattle. No onset of immunity is established or proposed in cattle. Whilst according to available guidance for Art. 25 applications for IVMPs, definition of the onset of immunity after the primary vaccination schedule is a crucial parameter to allow appropriate use under exceptional circumstances, an acceptable level of efficacy will be established on a case-by-case

basis using a benefit/risk approach taking into account the available data. Please refer to overall conclusions on Part 4.

The absence of establishment of the duration of immunity is acceptable, in line with available guidance for Art. 25 applications for IVMPs, provided that it is clearly indicated in the SPC (as is proposed in the SPC for Syvazul BTV 3).

A lack of data regarding maternal derived antibodies and their effect on IVMP efficacy is acceptable, in line with available guidance for Art. 25 applications for IVMPs, provided that a clear statement is included in the SPC (as is proposed in the SPC for Syvazul BTV 3).

No clinical studies have been submitted in the dossier. In line with guidance, clinical trials are not required. Data on previous use in the field (according to Article 110 (2)) was provided. It is noted that the use of Syvazul BTV 3 was approved under Article 110 (2) of Regulation (EU) 2019/6 in the Netherlands, Belgium and Germany from April 2024. Available pharmacovigilance data are presented in Part 3 of the dossier.

Pre-clinical studies

Dose determination

As discussed in the introduction, the amount of antigen included in the vaccine is $\geq 10^{6.9}$ $CCID_{50}$ per ml, based on pre-inactivation titres, and was selected according to previous experience with the BTV multi-strain vaccine. The proposed minimum dose is considered acceptable.

Onset of immunity

Sheep:

Two preclinical challenge studies were conducted in sheep with Syvazul BTV-3, the first study investigated the OOI after administration of a single dose (the proposed recommended schedule), and one investigated the OOI after administration of two doses separated by 3 weeks (not the proposed recommended schedule). For the first study, the applicant has taken account of Ph. Eur. 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera", Ph. Eur. 0062 "Vaccines for veterinary use" and Commission Delegated Regulation (EU) 2021/805 amending Annex II to Regulation (EU) 2019/6 of the European Parliament and of the Council. The first study was conducted at the Wageningen Bioveterinary Research (WBVR), the Netherlands, while the second study was conducted in two phases; the first phase (immunisation) was carried out at the facilities of AM Animalia, Spain and the second phase (challenge) was conducted at WBVR.

Study 01: Efficacy of two inactivated BTV-3 vaccine candidates against an experimental BTV-3 challenge in sheep

In this study, 24 seronegative, healthy non-pregnant ewes (breed: Swifter / Texelaar) approximately 11 to 12 months of age, not previously vaccinated against BTV and free from BTV, were allocated to three treatment groups (n=8/group). On Study Day (D) 0, treatment group T01 received a placebo (saline) and T02 and T03 received inactivated BTV-3 vaccine by subcutaneous injection. Four weeks post-vaccination (D28), sheep were challenged with BTV-3 at a dose of $4x\ 10^{4.8}$ $TCID_{50}$ in a 4 ml challenge dose by subcutaneous injection. During the follow-up period of 21 days post-challenge, rectal temperatures, clinical signs and lesions, viraemia, BTV antibodies and BTV 3-specific neutralising antibodies were evaluated. Animals that were euthanised due to compliance with predefined humane endpoint criteria

were subjected to necropsy. Remaining sheep were euthanised and subjected to necropsy at the end of the 21 day post-challenge study period (D49).

Results:

The challenge induced a severe infection in the control sheep; with 7/8 sheep reaching humane endpoints for euthanasia between days 8 and 13 post-challenge, of these on day 11 post-challenge one sheep had mostly recovered from some swellings and mucocutaneous lesions but was suffering with painful feet. This latter sheep was euthanised on day 13, and the remaining 1/8 sheep in the control group met scientific criteria for endpoint on the same day. In contrast, a reduction of mortality was observed in each of the vaccinated groups T02 and T03; 1/8 sheep in each of the groups T02 and T03 met scientific endpoint criteria on day 14 post-challenge (on the basis of continuing lameness accompanied with depression, the animal's current minimal contribution to the study outcome no longer justified its' compromised welfare at that stage of the experiment). Regarding the proposed claim for a prevention of mortality, it is considered that since the clinical signs which were considered to justify euthanasia in the control group T01 were remarkably similar to each of the two sheep in T02 and T03 that were euthanised, the data are considered to support a reduction in mortality, and not prevention, following challenge after vaccination with a single dose in sheep.

All sheep in both vaccinated groups developed clinical signs of disease following challenge including facial oedema, nasal discharge, cough, dyspnoea, depression, reduced appetite and three sheep in T02 and one sheep in T03 developed locomotory issues (lameness and /or leg disorders). Sheep in both vaccinated groups recovered from challenge during the 21 day follow-up period, apart from one sheep in each vaccinated group that met endpoint criteria as discussed above, and one sheep in T02 that had a score of 1 for dyspnoea on the last day of the study (but no other signs). A statistically significant reduction in the daily composite clinical score between the vaccinated groups T02 and T03 compared to the control group T01 on days 8, 9, 10, 11 and 12 post-challenge ($p \leq 0.05$) was demonstrated. Fever (rectal temperature > 40.0º C) was reported in all sheep from 4 days post-challenge for at least 4 consecutive days. Some statistically significant differences were reported however temperatures were higher in the vaccinated groups from days 4 – 6 post-challenge, then were higher in the control group from days 8 – 10 post-challenge. No positive effect of vaccination for a reduction of fever was evident. Although there was a breakthrough of clinical signs in vaccinated sheep (clinical signs typical of infection with BTV-3), the severity was reduced relative to controls and the majority of animals recovered. It is accepted that the data show that there was a reduction of clinical signs in the vaccinated groups compared to the control group.

A similar viraemic profile was observed in the three study groups. All sheep in each of the three groups rapidly became viraemic after challenge (from day 3 post-challenge onwards, and on day 5 when the highest levels of viraemia were observed, the mean cycle threshold (Ct) values were 19.9, 22.3 and 23.0 in groups T01, T02 and T03, respectively. Viraemia remained high at days 7 and 10 in all groups, with mean Ct values of 20.4, 22.6 and 23.3 in T01, T02 and T03, respectively, at day 7, and mean Ct values of 21.7, 23.8 and 24.8 in T01, T02 and T03, respectively, on day 10. Notwithstanding the similar viraemic profile in the study groups, a statistically significant difference between groups is reported on days 5, 7 and 10 for mean Ct values between T01 compared to T02 and T03, demonstrating a reduction in viraemia in the two vaccinated groups.

In both vaccinated groups, seroconversion as measured by antibodies against BTV VP7 was evident at 14 days post-vaccination in the majority of animals (7/8 in each group). However, 2 weeks later on the day of challenge, only 4/8 sheep in T02 and 2/8 sheep in T03 were seropositive which does not appear to be indicative of a strong persistent immune response to vaccination. One week post-challenge, all sheep in T02 and T03 were seropositive.

All sheep in both T02 and T03 were negative for virus neutralising antibody titres at four weeks post-vaccination (D28). At one week post-challenge (D35), 1/8 and 3/8 sheep in T02 and T03, respectively, had started to develop neutralising antibodies and by 3 weeks post-challenge, all tested animals in T02 and T03 were seropositive for neutralising antibodies. Therefore, the data demonstrate that the administration of a single dose of Syvazul BTV3 under the conditions of this study, does not stimulate the production of detectable neutralising antibodies against BTV-3.

Post-mortem analysis showed lesions consistent with BTV infection and were more prominent in sheep in the control group than sheep of the vaccinated groups.

The CVMP notes that this study was not performed in sheep of the proposed minimum age, however this deviation from requirements / identified data gap can be accepted since the candidate product is closely related to Syvazul BTV vaccine (which is authorised for use in sheep and cattle of the same proposed minimum age as Syvazul BTV 3) and significant (age-dependent) differences would not be expected on the basis of inclusion of serotype 3 in Syvazul BTV 3 vs BTV serotypes 1, 4 or 8 in the same vaccine formulation. In addition, the second challenge study in sheep has been conducted in sheep of approximately 3 – 4 months of age.

Overall, it is concluded that the data provided in this study support a positive effect of vaccination for a reduction of mortality and viraemia. Further, although there was a breakthrough of clinical disease in vaccinated animals following challenge at 28 days after the administration of a single dose, clinical signs were less severe and a statistically significant reduction in clinical signs was demonstrated, therefore it is accepted that a reduction of clinical signs and lesions has been demonstrated.

Study 02: Serological efficacy study following administration of two BTV-3 vaccine candidates to lambs & Study 03: Efficacy of a prime-boost administered BTV-3 vaccine against experimental BTV infection in sheep

The second study conducted in sheep was carried out as two different studies conducted by different clinical or contract research organization (CROs); the first study, Study 02**,** corresponded to the immunisation phase and the second study, Study 03, corresponded to the challenge phase. Thirty seronegative healthy lambs (breed: Lacaune / Ripollesa) approximately 3 to 4 months of age, born to non-vaccinated ewes, were allocated to three treatment groups (n=12 for vaccine groups, n=6 for control group). On Study Day (D)0 and 28, animals in treatment groups 1 and 2 received two different batches of inactivated BTV-3 vaccine (different batches with the same formulation) and animals in the control group were mock-vaccinated (PBS) by subcutaneous injection in the axillary area (behind the right axillary area for the first dose, and in the left axillary area for the second dose). At 15 days after the second dose, 10 vaccinated animals belonging indistinctly to vaccine groups 1 and 2 were randomly selected and transferred to the challenge facilities, together with all animals of the control group. From then on, only two groups were considered in the study: one vaccinated group (referred as T02) and the control group (T01). At 30 days after the 2nd dose (D58), lambs were challenged with BTV-3 at a dose of 4x 10^5 $TCID_{50}$ by subcutaneous injection. During the follow-up period of 21 days post-challenge, rectal temperatures, clinical signs, viraemia, BTV antibodies and BTV-3 specific neutralising antibodies were evaluated. The in-life phase of the study ended on D79 (21 days post-challenge), when sheep were euthanised and subjected to necropsy.

Results:

The results of this study showed that the challenge did not result in severe clinical signs of disease or mortality / reaching of humane end points in any sheep with mild clinical signs of disease only manifesting in each group. Nonetheless, all animals developed fever which was worse in the vaccinated group compared to the control group, as evidenced by statistically significantly higher temperatures in the vaccinated group on days 4, 5 and 6 post-challenge. The applicant reports a statistically significant

difference in the daily clinical composite score in the control group (higher in the control compared to the vaccinated group), however it is not considered that a clinically relevant reduction of clinical signs of disease has been demonstrated in this study, since clinical signs were generally insufficiently severe to allow robust comparisons between vaccinated and control lambs.

Whilst all vaccinated animals became viraemic, there was a significant reduction in the level of viraemia in the vaccinated group compared to the control group with statistically significant differences in the mean Ct values on days 7, 10, 12, 14, 17, 19 and 21 post-challenge.

Seroconversion as measured by ELISA antibody titres at 21 days after the administration of the first dose was reported in 8/12 vaccinated lambs, however one week later 3 of the previously seropositive 8 lambs were seronegative. Administration of the second dose resulted in 100% of lambs (12/12) seropositive at one week after completion of the vaccination scheme.

Neutralising antibodies developed after the second dose of vaccine, but did not persist. While neutralising antibodies were detected at 1 week after the administration of the second dose of vaccine, by two weeks later on day of challenge, they had decreased to undetectable levels. After challenge, neutralising antibodies were detected in both the control and vaccinated groups.

No BTV-3 related lesions were reported at post-mortem in either group.

There are no data provided in this study which support the benefit of administering a second dose to sheep, since the OOI tested at 30 days after the completion of the two-dose basic vaccination scheme in sheep does not demonstrate that this scheme is more effective than a single dose basic vaccination scheme. This may be due to the fact that only mild clinical signs of disease were induced by challenge (despite using the same challenge inoculum and titre as was used in the first challenge study conducted in sheep) such that a clear difference in effect between vaccinated and unvaccinated sheep is not observed. The only relevant differences between this study and the previous study are the vaccination schedule and the age of animals. For reasons unknown, lambs in this study did not succumb to challenge compared to the severe signs of disease which were induced in 11 – 12 month old sheep in the first study. The applicant also discusses this point, noting that an explanation of the observed difference between challenges has not been identified so far (differences for the challenge material have been ruled out). Moreover, viraemia was detected in all control (and vaccinated) sheep. Differences identified in the present study are mostly related to the animals included and consist of the age (young sheep aged 6 months vs. 12-13 months at challenge), sex (male and female vs. only female) and breed (Lacaune × Ripollesa vs. Swifter × Texelaar).

Overall, it is concluded that the data from this study provide only limited support for a positive effect of vaccination for the proposed claims. No benefit of vaccination for protection against clinical signs and lesions could be reliably demonstrated in the absence of a sufficiently severe challenge. For the parameter pyrexia, there was no clear and consistent difference between groups. At necropsy, no lesions were observed in any animals. However, the data demonstrate that whilst all vaccinated sheep became viraemic and had a similar viraemia profile to mock-vaccinated sheep, there was a statistically significant reduction of viraemia in the vaccinated group.

Cattle:

One study is presented, which investigated the serological response to vaccination when calves of minimum age were administered two doses of Syvazul BTV 3, separated by an interval of 21 days, intramuscularly, in accordance with the recommended conditions of use. The animal phase of the study was carried out in the facilities of AM Animalia (La Vall de Bianya, Girona, Spain) where cattle were vaccinated and maintained until the end of the study.

Study 04: Serum antibody efficacy study following administration of two BTV-3 vaccine candidates to cattle.

In this study, thirty healthy unvaccinated male calves (breed: Friesian Holstein cross-breed) approximately 2 months of age, non-vaccinated against any BTV serotype and negative to BTV as tested by RT-qPCR. Sixteen of 30 animals were seropositive to VP7 BTV antibodies by ELISA at arrival, considered to be maternally derived. Calves were randomly distributed to four treatment groups; three vaccinated groups (n=8 /group) and one placebo control group (n=6). On Study Day (D)0 and 21, groups 1, 2 and 3 received the vaccine. Group 4 were mock-vaccinated (4 ml PBS). Test and control articles were administered by intramuscular injection on the right side of the neck for the first dose, and on the left side of the neck for the second dose. After vaccination, calves were monitored for 21 days after the second dose (until D42) with collection of blood samples on D0 D21, D28, D35 and D41 to measure the serological response to vaccination, evaluated by testing at laboratorios Syva serum VP7 BTV antibodies and a BTV3 specific VN antibody response after each vaccination, as assessed by ELISA and VN test, respectively.

Results:

Antibodies against VP7 of BTV were present 12 days prior to vaccination in 16/30 calves, with the levels decreasing between that timepoint and the day of vaccination in all groups. On day 0, 50%, 38%, 50% and 33% of calves included in group 1, 2, 3 and control group 4, respectively, were seropositive. Mean levels remained similar or marginally lower at D21; no seroconversion was observed after the first dose in the vaccinated groups. Overall, mean titres in the control group decreased between D-12 to D41. One week after the second dose (D28), a serologic response was detected in each vaccinated group, At D28, 75% (6/8), 88% (7/8) and 75% (6/8) of calves in groups 1, 2 and 3 were seropositive, respectively. At 2 and 3 weeks after the second dose, the antibody response gradually increased in group 1, whereas for group 2 a slight decrease in mean levels followed by an increase was observed. Mean titres were consistently higher in group 2 compared to group 1, whereas for group 3, the proportion of animals that were seropositive remained the same until the end of the study. Overall, it can be concluded that a serological response to vaccination is highest in group 2, with a slightly lower response in group 1, and an inadequate serological response in group 3 (lower antigenic payload). The serological response was not evident after the first dose but was clearly induced after administration of the second dose.

The results of the virus neutralisation test showed that on D0, all animals were seronegative to BTV3 neutralising antibodies. In the vaccinated groups, neutralising antibodies developed in all three groups to a similar extent and were present at the first timepoint evaluated after vaccination at Day 21, prior to administration of the second dose, in the majority of vaccinated animals (75%, 88% and 75% of groups 1, 2 and 3, respectively). One week after the second dose, and on each of the subsequent two weeks, neutralising antibodies were detected in 88% of calves in each group. The mean VN titres do not appear to be correlated with the antigenic payload. It is of concern however, that neutralising antibodies were detected in 2 of 6 calves in the control group. These data could suggest a level of infection pressure in the animal facilities, however it is acknowledged that BTV-3 was confirmed absent in study animals by RT-qPCR on days -8 and day 0 (first dose). Regarding the results in the placebo group, the applicant acknowledges that "2 calves inconsistently tested positive. The remaining 4 animals were negative until the end of the study." This, in the applicant's view, infers that the results for these two calves were artifacts.

Group 1 are considered a slightly worse case scenario as only half the amount of adjuvant of a standard dose was administered in the lower volume dose. Group 3 were vaccinated with only half of the proposed immunising dose and adjuvant.

It is concluded that the data provided in this study would suggest an active immune response, in terms of a demonstrable antibody response to vaccination after the second dose. Whether that would correlate

with protection from infection at that time is unknown. ELISA titres are considered supportive and serve as a general indicator of serological response, whereas neutralising antibodies against BTV-3 are considered more vaccine-specific and are more closely related to a biological effect. Please refer to overall conclusions on Part 4.

Applicant's extrapolation of data from sheep to cattle and justification of neutralising antibodies as surrogate of protection

In addition to the serological study in cattle, the applicant has provided a comparison of performance of Syvazul BTV 3 in sheep and cattle in order to justify that the neutralising antibodies are a relevant marker of protection, by extrapolating data from the sheep challenge studies, in addition to citing bibliographic data to support this position.

To compare performance of the vaccine in sheep and cattle, the applicant compares data from the study in which sheep were vaccinated (two-dose scheme) with two vaccines that were identical to the vaccine 2 used in the cattle study. When BTV3 neutralising antibodies at day 42 post vaccination were compared between these sheep and the calves in the cattle serological study, it can be observed that the GMT were higher in the calves than in sheep This was also evident when the frequency of the individual titres observed in calves at day 41 post vaccination were compared with that in sheep at day 42 post vaccination.

The applicant also considers that neutralising antibodies against the virus have been used as the most suitable surrogate indicator of protection against BTV in vaccinated animals (e.g. Oura et al., 2009; Letchworth and Appleton, 1983; Zanella et al., 2013, Sailleau et al. 2022). Although a definitive correlation that permits the establishment of threshold levels for protection cannot be found, it is argued that neutralising antibodies are part of the functional immune response that protects animals against the infection and/or the disease. Thus, this indicator can be considered the most useful tool available to compare the effect of vaccination, apart from the challenge of animals (Zanella et al., 2013, Sailleau et al. 2022). The applicant considers that the response of BTV3 neutralising antibodies in calves after vaccination was not lower than the response obtained in sheep that were protected against challenge (reduction of viraemia, reduction of clinical signs and lesions). This response was generated in calves using vaccines equal or with a lower content on active substance and/or adjuvants. Therefore, the use Syvazul BTV 3 in calves could be allowed in absence of challenge experiments, by extrapolation from data obtained in sheep, based on the comparison of the neutralising antibody response elicited in the two species, that is even higher in cattle.

This is also stated to be in agreement with studies performed with Syvazul BTV for the serotypes BTV-1, BTV-4 and BTV-8, which demonstrated that all the vaccines that were efficacious in sheep were also able to protect cattle.

In the CVMP's view, while these data are noted, it is not possible to conclude if extrapolation from sheep to cattle is a valid approach for BTV serotype 3 given the differences in the pathogenesis of disease in the two target species, where clinical signs would be expected to milder (or subclinical) in cattle compared to sheep. Furthermore, noting the limitations of the study presented in sheep after the two-dose vaccination scheme, the data from that study are considered to provide only limited support the claimed clinical protection in sheep. As acknowledged by the applicant, a strong correlation between serology and protection from BTV has not been established, however the development of neutralising antibodies may serve as a more general indicator of response to vaccination. However, no challenge data were available to demonstrate efficacy in calves at time of submission of the marketing authorisation procedure. Please refer to overall conclusions on Part 4.

In line with the Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances EMA/CVMP/IWP/251947/2021, "*Efficacy should be demonstrated in laboratory conditions by a challenge model in all recommended target species and categories recommended for vaccination unless scientific/literature data can be provided demonstrating that extrapolation from one species to another species or from one category of a species to another category of the same species is possible*."

In this case argumentation is presented to extrapolate that neutralising antibodies in one species (sheep) would be protective in another species (cattle) at the same titre, but there is no scientific data/literature data itself which would support this point.

Overall, the validity of the approach of the comparison of neutralising antibody titres in sheep in a pre-clinical study (using the same batch of vaccine, and a two dose scheme) to that of the neutralising antibody titres in calves not subjected to challenge in this dossier is not supported. Notwithstanding that for Art. 25 applications, an *acceptable level of efficacy will be established on a case-by-case basis using a benefit/risk approach taking into account the available data*, no challenge data are available to support that the presence of neutralising antibodies in cattle following vaccination with Syvazul BTV 3 is correlated with protection from clinical signs of disease.

CVMP overall conclusions on onset of immunity

Sheep:

Following the administration of a single dose to sheep, efficacy was investigated at 28 days post-vaccination. A severe challenge was induced using a homologous BTV-3 challenge strain. The data supported a positive effect of vaccination for a reduction of mortality. Whilst there was a breakthrough of clinical signs in vaccinated sheep, a reduction of clinical signs and lesions and a reduction of viraemia in the vaccinated groups was evident compared to the control group. Furthermore, it is noted that the 2024 pandemic of BTV-3 is strongly associated with high mortality in sheep, and this is considered the pivotal parameter for which protection is required.

Following the administration of a two-dose vaccination scheme to sheep, separated by an interval of 28 days, efficacy was investigated at 30 days after completion of the scheme in approximately minimum age lambs. Using a homologous BTV 3 challenge strain and similar conditions as for the first study, only a mild challenge was induced. The results of this study provide only limited support for a positive effect of vaccination for the proposed claims. No benefit of vaccination for protection against clinical signs and lesions could be reliably demonstrated in the absence of a sufficiently severe challenge. However, as for the previous study, whilst all vaccinated sheep became viraemic and displayed a similar viraemia profile to mock-vaccinated sheep, a reduction of viraemia was demonstrated in the vaccinated group.

It is unfortunate that a more robust efficacy profile could not be demonstrated after the administration of a two-dose scheme in sheep compared to a single dose scheme, since from an immunological perspective, a more robust response to vaccination would be expected following second exposure of the immune system to an adjuvanted, inactivated vaccine. Safety data are available in sheep concerning the safety of a two-dose scheme (see Part 3).

It was noted that pharmacovigilance data submitted with this application (see Part 3) indicate reports of LEE following administration of a single dose to sheep in EU member states affected by the BTV-3 outbreak where Syvazul BTV-3 has been used under special licence. Further, based on publicly available information, the CVMP is aware that the Dutch Veterinary Medicines Agency noted that a number of reports of suspected lack of efficacy in sheep were reported, and whilst there were no indications that the benefit risk balance needed to be adjusted, the minister made a decision that veterinarians may

deviate from the SPC and administer a second vaccination, the so-called booster vaccination, to sheep. While the two-dose study presented by the applicant did not provide evidence of added benefit of the second dose, the applicant was requested to comment further on the adequacy of protection after the administration of a single dose. and, when addressing this question, was asked to consider whether or not, based on field experience (i.e., use in accordance with Art. 110(2) of Regulation (EU) 2019/6), there was evidence to support added benefit of a two-dose vaccination schedule. The applicant provided a discussion and summary of available field data including LEE data following vaccination with a single dose vs two dose scheme. The data available to the applicant was limited in terms of conclusions that could be drawn. Based on the laboratory data provided, the proposed recommended single dose scheme was considered acceptable.

In conclusion, the data provided were considered to support a reduction of viraemia, mortality, clinical signs and lesions following use in accordance with the recommended vaccination schedule (single dose).

Cattle:

Following the administration of a two-dose scheme, in accordance with the recommended conditions of use, a serological response to vaccination was achieved for both ELISA antibody titres against VP7 of BTV (not serotype-specific) and BTV 3 neutralising antibodies.

However, the use of neutralising antibodies as a surrogate of efficacy is not accepted in the absence of a dedicated study comparing protection from challenge and neutralising antibody titres in calves with Syvazul BTV 3.

In the absence of challenge data to confirm that active immunisation as evidenced by stimulation of neutralising antibodies would be correlated with efficacy following challenge, the CVMP considered that a claim for active immunisation against bluetongue virus serotype 3 was supported, however insufficient data were available to demonstrate that this was correlated with protection. Thus, the claim for active immunisation was not accepted since it was not considered to represent a clinically meaningful claim. In light of these data gaps, which were not considered acceptable by CVMP, the applicant withdrew cattle as target species for Syvazul BTV 3.

In line with relevant guidance, "Where a veterinary medicinal product has been granted a marketing authorisation in accordance with Article 25 of Regulation (EU) 2019/6, the SPC shall clearly state that only a limited assessment of quality, safety or efficacy has been conducted due to the lack of comprehensive data." Therefore, the following statement is included in the SPC:

"Marketing authorisation in exceptional circumstances and therefore assessment based on customised requirements for documentation. Only a limited assessment of quality, safety or efficacy has been conducted due to the lack of comprehensive quality, safety or efficacy data."

Apart from the data provided in support of use in cattle, which were not considered adequate, the data gaps outlined above for use in sheep are considered to represent acceptable deficiencies in the context of an application for authorisation in accordance with Article 25 (authorisation in exceptional circumstances).

Duration of immunity

No data provided. Refer to introductory comments. Whilst the lack of data to support a duration of immunity is considered an acceptable data gap for an Article 25 application, in the absence of data to support the proposed revaccination schedule of a single dose after 12 months, the applicant was requested to omit this recommendation and to replace with the text:

"Revaccination:

Not established."

However, the applicant is requested to provide data to support a duration of immunity in the future as a specific obligation.

Maternally derived antibodies (MDA)

No data provided. Refer to introductory comments. The statement included in the SPC "No information is available on the use of the vaccine in sheep with maternally-derived antibodies." is considered appropriate.

Interactions

No data provided. The standard statement proposed for inclusion in section 3.8 of the SPC is considered appropriate; "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."

Clinical trials

No data provided. The absence of clinical trials is acceptable in principle, provided that adequate reassurances are available from pre-clinical studies to support that a reasonable level of efficacy will be achieved.

Please refer to overall conclusions on Part 4.

Overall conclusion on efficacy

Pre-clinical studies are presented in support of efficacy of proposed claims for Syvazul BTV 3.

The onset of immunity was investigated in two challenge studies in sheep and was investigated in cattle by the evaluation of the serological response to vaccination.

The BTV-3 antigen input of batches used in the studies in sheep was $10^{7.2}$ $CCID_{50}$/. Batches formulated with an antigen input of $10^{7.2}$ $CCID_{50}$/ml represent two times the fixed target antigen amount compared to a batch formulated with the proposed minimum fixed target antigen amount. Although no correlation between the potency of the batches with clinical efficacy is possible (no potency test is currently available and an interim potency test is being used based on quantification of viral titre pre-inactivation), this is an acceptable approach in accordance with the legal basis of the application. As discussed in Part 4.A, the use of a non-minimum antigen content batch is an data gap / acceptable derogation from Annex II requirements for the efficacy studies, in accordance with the Guideline on data requirements for authorisation of immunological veterinary medicinal products in exceptional circumstances (EMA/CVMP/IWP/251947/2021) where it is stated "*For inactivated IVMPs, the use of standard production batches (it is not required to use a batch of minimum antigen content) is possible in efficacy studies. The safety and efficacy studies may be combined in the same pre-clinical (laboratory) study, using the same batch(es) of an IVMP.*"

In order to support the efficacy of vaccination in sheep, two pre-clinical studies involving challenge are provided; the first investigating efficacy after the proposed scheme, the second after a two-dose vaccination scheme whereby two doses were administered separated by an interval of 4 weeks. In the first study, following a severe challenge, the data supported a positive effect of vaccination for a reduction of mortality and viraemia. A reduction of clinical signs and lesions in the vaccinated groups was shown,

although some breakthrough of clinical signs of BTV 3 challenge in vaccinated sheep was observed. However, it is noted that the 2024 pandemic of BTV-3 is strongly associated with high mortality in sheep, and this is considered the pivotal parameter for which protection is required. Due to an unexpectedly mild challenge in the challenge study following the administration of two doses, the data provide only limited support for a positive effect of vaccination for the proposed claims. In conclusion, the data were considered to support a reduction of viraemia, mortality, clinical signs and lesions following use in accordance with the recommended vaccination schedule (single dose). This conclusion was based on the data package available to CVMP, taking into account the legal basis of the application (Article 25) and noting that efficacy for such applications will be determined on a case-by-case basis in the framework of limited available efficacy data.

Efficacy in cattle was investigated in one pre-clinical study only by the determination of the serological response to vaccination. ELISA antibody titres to BTV (not serotype-specific) demonstrates an immunological response to vaccination at 2 – 3 weeks after the second dose. BTV-3 specific virus neutralising antibodies were measured, which is considered a more robust measure of the immune response to vaccination than ELISA titres given the lack of challenge data in cattle. Vaccination according to the proposed scheme was shown to stimulate the production of neutralising antibodies. However, the presence of neutralising antibodies are not definitively correlated with protection and can only serve as indicator of vaccine take. In the absence of challenge data to confirm efficacy of Syvazul BTV 3 in cattle, it was considered that insufficient data were available to support the indications for use, therefore the applicant withdrew cattle as proposed target species.

No data are provided concerning duration of immunity, or the potential impact of MDAs on vaccine efficacy. This is clearly indicated in the proposed SPC and can be accepted for an application submitted under Article 25 of Regulation (EU) 2019/6. The proposed revaccination schedule of revaccination with a single dose after 12 months was not considered to have been adequately supported. Therefore, the SPC states "Revaccination: not established." However, data to support duration of immunity should be provided as a specific obligation by February 2026.

The applicant proposes to include the following text in section 4.1 of the SPC "To stimulate active immunity of sheep against bluetongue virus serotype 3". The text is considered acceptable.

No clinical studies are presented in the dossier. This is acceptable in principle noting the legal basis of the application; specific derogations for efficacy requirements are permitted for applications submitted in accordance with Art. 25).

Part 5 – Benefit-risk assessment

Introduction

Syvazul BTV 3 is a suspension for injection containing ≥ $10^{6.9}$ $CCID_{50}$ (the ($CCID_{50}$) 50 % cell culture infective dose determined before inactivation) of Bluetongue virus, serotype 3, BTV-3/NET2023, inactivated and is presented in cardboard box with one polypropylene colourless vial containing 80 ml or 200 ml.

The active substance of Syvazul BTV 3 is Bluetongue virus, serotype 3, BTV-3/NET2023, Inactivated, as active substance and contains two adjuvants (Aluminium hydroxide and Purified saponin (Quil-A) from *Quillaja saponaria*). The target species were initially proposed as Cattle and Sheep. The route of administration is subcutaneous in sheep and intramuscular in cattle.

At the time of submission, the applicant applied for the following indications:

"Sheep: for active immunisation of sheep to reduce viraemia, to prevent mortality and reduce clinical signs and lesions caused by bluetongue serotype 3.

Cattle: for active immunisation against bluetongue virus serotype 3."

The recommendation for administration in sheep from 3 months of age with a single 2 ml dose primary vaccination and a revaccination with one dose of 2 ml after 12 months.

The initial recommendation for administration in cattle was from 2 months of age in naïve animals or from 3 months of age in calves born to immune cattle with two doses of 4 ml 3 weeks apart and a revaccination with one dose of 4 ml after 12 months.

The application has been submitted in accordance with Article 25 of Regulation (EU) 2019/6 in exceptional circumstances. Reduced data requirements therefore apply and have been considered in the assessment. These reductions relate to quality, safety and efficacy.

Benefit assessment

Direct benefit

It is considered that the direct benefit of this vaccine is clear, since availability of the vaccine would meet a currently unmet need for a BTV serotype 3 vaccine which is needed based on the current epidemiological situation in the EU.

For the target species sheep, the benefit of Syvazul BTV 3 is its efficacy for the claims: a reduction of viraemia, mortality, clinical signs and lesions.

For the target species cattle, whilst the claimed benefit of Syvazul BTV 3 is its efficacy for the claim 'for active immunisation against bluetongue virus serotype 3', in the absence of challenge data to confirm that active immunisation, as demonstrated by the presence of neutralising antibodies, could be correlated with vaccine efficacy, it was concluded that insufficient data were provided to support this claim. The direct benefit of Syvazul BTV 3 in cattle was not considered to have been adequately supported, therefore this target species was removed from the SPC.

Additional benefits

Syvazul BTV 3 increases the range of available treatment possibilities for vaccination against BTV serotype 3.

Risk assessment

Quality

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner under exceptional circumstances. The results of tests carried out indicate a level of consistency and uniformity of important product quality characteristics in accordance with Article 25 requirements, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Additionally, specific obligations as post-authorisation measures to the marketing authorisation under

exceptional circumstances are established:

- The applicant should provide the completion of the development of the in vitro potency test for the BTV3 antigen (specific obligation).

-The applicant is requested to provide data from one industrial scale batche to confirm the proposed shelf life and the recommended storage conditions for the BTV 3 antigen and the Syvazul BTV 3 finished product (specific obligation).

Safety

Risks for the target animal

Administration of 'Syvazul BTV-3' in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include an increase in temperature and injection site reactions.

Risk for the user

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice is included in the SPC.

Risk for the environment

'Syvazul BTV 3' is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

'Syvazul BTV 3' is not expected to pose a risk for the consumer. The withdrawal period is zero days.

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 animal, user, environment and consumer, and to provide advice on how to prevent or reduce these risks.

User safety

No specific user safety risks have been identified. General user safety warnings have been included in the SPC.

Environmental safety

No specific risks to the environment have been identified. Standard advice on waste disposal is included in the SPC.

Conditions or restrictions as regards the supply or safe and effective use of the VMP concerned, including the classification (prescription status)

The veterinary medicinal product is subject to a veterinary prescription.

Post-authorisation measures

Two Quality and one efficacy post-authorisation measures are identified and are considered justified in line with the application under Article 25 'Exceptional circumstances'.

Specific obligations to complete the post-marketing authorisation measures for the marketing authorisation under exceptional circumstances are detailed in Annex II of the product information and mentioned below.

Description	Due date
Completion of the development of the in vitro potency test for the BTV 3 antigen.	January 2026
Data from the completed stability study should be provided to confirm the proposed shelf life and the recommended storage conditions for the inactivated BTV 3 antigen and the Syvazul BTV 3 finished product.	January 2027
A study on duration of immunity in sheep should be conducted and data should be provided as soon as available.	February 2026

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indications:

"Sheep: for active immunisation of sheep to reduce viraemia, to prevent mortality and reduce clinical signs and lesions caused by bluetongue serotype 3.

Cattle: for active immunisation against bluetongue virus serotype 3."

The following claims are considered to have been adequately supported:

Sheep: for active immunisation of sheep to reduce viraemia, mortality, clinical signs and lesions caused by bluetongue virus serotype 3.

Based on the data presented to date, the overall benefit-risk balance is considered positive.

As the application was submitted under Article 25, certain pivotal data on quality, safety and efficacy were not included in the dossier. However, the CVMP considered that the overall benefit of the availability of the veterinary medicinal product would outweigh the risk of absence of these data, also taking into consideration the risk management measures addressed above.

The product information has been reviewed and is considered to be satisfactory and in line with the assessment.

Conclusion

Based on the original data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) considers that Syvazul BTV 3 was approvable since these data satisfy the requirements for an authorisation set out in the legislation in accordance with Article 25 (Regulation (EU) 2019/6).

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.

In addition, based on the review of data on the quality-related properties of the active substance bluetongue virus, serotype 3, BTV-3/NET2023, inactivated, contained in the veterinary medicinal product Syvazul BTV 3, the CVMP considers that the active substance is not to be qualified as a new active substance considering that another vaccine which contains inactivated bluetongue virus, serotype 3 was granted a marketing authorisation in the EU in October 2024.