6 December 2018
EMA/870927/2018
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Zulvac BTV to add cattle as a new food-producing target animal species (EMEA/V/C/004185/X/0001)

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 Zoetis Belgium SA submitted on 28 September 2017 an application to the European Medicines Agency (the Agency) for a line extension to the marketing authorisation of Zulvac BTV (formerly Zulvac BTV Ovis), through the centralised procedure under Article 19 of Commission Regulation (EC) No. 1234/2008 and Annex I thereof.

Zulvac BTV Ovis is a bluetongue virus (BTV) vaccine (inactivated) (multi-strain: 1 strain out of a set of 3) authorised for the active immunisation of sheep by the subcutaneous route of administration and contains inactivated bluetongue virus, serotype 1, serotype 4 or serotype 8. Zulvac BTV Ovis is an immunological veterinary medicinal product and was authorised for use in the European Union on 25 April 2017.

This extension application is to add a new target species, cattle. As a consequence of the extension procedure the name of the product is changing from Zulvac BTV Ovis to Zulvac BTV.

For cattle, the vaccine will contain a maximum of one of two BTV serotypes (serotype 1 or serotype 8) and is intended for the following indication:

For active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus (BTV), serotype 1 or serotype 8.

Onset of immunity:

- 15 days after completion of the primary vaccination scheme for BTV-1

- 25 days after completion of the primary vaccination scheme for BTV-8

Duration of immunity:

- 12 months after completion of the primary vaccination scheme for BTV-1

- 12 months after completion of the primary vaccination scheme for BTV-8

The vaccine is presented as a suspension for injection and administered as a 2 ml dose. It is filled into high density polyethylene (HDPE) bottles of 10, 50 and 120 doses which are closed with chlorobutyl elastomer stoppers and aluminium seals.

The rapporteur appointed is Rory Cooney and the co-rapporteur is Frédéric Klein.

The dossier has been submitted in accordance with Article 19 of Commission Regulation (EC) 1234/2008 and Annex I thereof (extensions).

On 6 December 2018, the CVMP adopted an opinion and CVMP assessment report.

On 21 February 2019, the European Commission adopted a Commission Decision granting the extension to the marketing authorisation for Zulvac BTV.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Multi-strain dossier

The application has been submitted in accordance with the revised Annex I to Directive 2001/82/EC and for which the CVMP has published guidance (EMA/CVMP/IWP/105506/2007) on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD). Three strains of BTV are included in the dossier, namely BTV serotypes 1, 4 and 8. The vaccine may contain up to 1 type of strain of inactivated BTV antigen from the 3 strains included in the dossier when intended for sheep or up to 1 strain of inactivated BTV antigen (serotype 1 or serotype 8 only) when intended for cattle, depending on epidemiological need.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated 2 May 2017) which fulfils the requirements of Directive 2001/82/EC, as amended. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

The manufacture of the active substance and the finished product, including all packaging, and batch release is carried out by Zoetis Manufacturing & Research Spain S.L. (Girona, Spain).

The site is routinely inspected by EU regulatory authorities and has been inspected within the last three years by the Spanish Agency of Medicines and Medical Devices (dated 27 February 2017) and a valid Good Manufacturing Practice (GMP) certificate is available (dated 28 May 2017). No additional inspections specific to this vaccine are considered necessary.

Overall conclusions on administrative particulars

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

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

Part 2 - Quality

Composition

The finished product is presented as a suspension for injection and may contain one of the following inactivated BTV strain antigens as active substance, at concentrations equivalent to RP per dose (RP=relative potency by a mice potency test compared to a reference vaccine efficacious in cattle (BTV-1 and 8) and sheep (BTV-1, 4 and 8)) as follows:

BTV, serotype 1, strain BTV-1/ALG2006/01 E1: RP ≥ 1 (at release and end of shelf life)

BTV, serotype 8, strain BTV-8/BEL2006/02: RP ≥ 1 (at release and end of shelf life)

BTV, serotype 4, strain SPA-1/2004: RP ≥ 1 (at release) and RP ≥ 0.8 (at end of shelf life)

The vaccine contains aluminium hydroxide and *Quillaja saponaria* saponin extract (Quil A) as adjuvants, thiomersal as preservative as well as other excipients, including sodium chloride, potassium chloride, disodium phosphate dihydrate, potassium dihydrogen phosphate and water for injections.

Container

The vaccine is presented as 25 ml (10 doses), 100 ml (50 doses) and 250 ml (120 doses), filled into high-density polyethylene bottles closed with chlorobutyl elastomer stoppers and subsequently sealed with an aluminium cap. The bottles and stoppers all meet pharmacopoeial standards.

Development pharmaceutics

Zulvac BTV has been developed for cattle and sheep in accordance with the multi-strain dossier concept introduced in the revised Annex I to Directive 2001/82/EC and is a line extension of the product intended for sheep only, Zulvac BTV Ovis. The three strains of BTV (only two for cattle) that may be incorporated separately into the finished product depending on epidemiological need have been selected based on expert advice and considering the epidemiological situation of bluetongue in the EU in 2015. A reasonable justification was given regarding the relevance of the chosen vaccine strains within the EU. Whilst the inclusion of the strains in principle is in line with the CVMP Guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) (EMA/CVMP/IWP/105506/2007), the product has not been developed in terms of being a flexible combination of 2 of the 3 proposed strains. The potential cross-reactions between strains have not been investigated and, consequently, only monovalent vaccines can be formulated under the current multi-strain dossier. The vaccine is adjuvanted with aluminium hydroxide and *Quillaja saponaria* saponin extract (Quil A) selected for its ability to stimulate immunity whilst keeping an acceptable safety profile in the target species (sheep and cattle).

The target quantities of each of the respective antigens per dose have been determined on the basis of the results of a number of safety and efficacy studies carried out in the target species both with monovalent vaccines and bivalent vaccines. The specifications for the antigen content have been revised for use in cattle and have a narrower range for BTV-1 and BTV-8 than previously agreed for the predecessor product (Zulvac BTV Ovis) intended for use in sheep only. The monovalent Zulvac BTV-4 vaccine is only proposed for use in sheep, therefore specifications and parameters are not

impacted by the addition of cattle as a new target species. Some developmental batches were formulated using antigen produced in bioreactors, however the applicant provided a satisfactory justification for the relevance of these data to support product produced in roller bottles.

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

Batches used in the clinical trials were formulated according to the method proposed for the marketed product.

Description of the manufacturing method

The method of manufacture remains the same as that agreed during the authorisation of the predecessor product Zulvac BTV Ovis except the antigen content at the bulk vaccine stage. The vaccine is manufactured by a fairly standard process which is identical for all the three different BTV strains. The BTV strains are cultured in baby hamster kidney cells (BHK-21) in roller bottles and inactivated by treatment with binary ethylenimine (BEI). After inactivation, the residual inactivant is neutralised with sodium thiosulphate. Antigen bulks can be stored at 2 °C – 8 °C for a maximum of 12 months. Data to support the storage of the bulk antigen for this period of time have been presented.

The option to produce BTV antigens in bioreactors, which was the subject of an existing post-authorisation measure, has been removed from the marketing authorisation dossier. Annex II (D) has been amended accordingly.

To formulate the finished product, the selected BTV inactivated antigen is mixed with the adjuvants and excipients. Maximum and minimum antigen inputs at formulation for each of the BTV antigens have been established based on the safety and efficacy studies, respectively. The calculation of the antigen input is based on virus titres pre-inactivation taking into account the dilution factor after inactivation and neutralisation. A blending table which can be applicable to any batch of monovalent vaccine was provided.

Whilst the manufacturing process remains unchanged, the applicant had originally proposed changes to the antigen content at formulation. The applicant originally proposed to have different specifications for BTV-8 antigen content for batches of product intended for use in sheep only ($10^{6.5}$ to $10^{8.1}$ $TCID_{50}$/dose) and batches intended for use in both sheep and cattle ($10^{7.3}$ to $10^{7.4}$ $TCID_{50}$/dose). The applicant's proposal was considered thoroughly and it was concluded that it presented a serious risk that a batch of Zulvac BTV-8 vaccine formulated for use in "sheep-only" could be used in cattle, for which safety and efficacy have not been demonstrated outside a narrow antigen content range ($10^{7.3}$ to $10^{7.4}$ $TCID_{50}$/dose). Consequently, the applicant agreed to set a single specification for BTV-8 antigen content that is safe and efficacious in both target species ($10^{7.3}$ to $10^{7.4}$ $TCID_{50}$/dose).

Production and Control of starting materials

Active substance

Detailed specifications have been provided for all starting materials used to manufacture the vaccine. The BHK-21 cell line and the various BTV master seeds are adequately tested to demonstrate freedom from extraneous viruses. Identity tests, based on RT-PCR and specific for each of the three BTV

serotypes included in the vaccine, have been introduced to confirm the identity of BTV master and working seeds.

Excipients

The aluminium hydroxide used as adjuvant complies with Ph. Eur. monograph 1664. The adjuvant Quil A is not listed in a pharmacopoeia but its in-house quality standard is satisfactory. All of the other excipients (thiomersal, sodium chloride, potassium chloride, disodium phosphate dihydrate, potassium dihydrogen phosphate and water for injections) comply with the respective Ph. Eur. monographs.

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

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

Control tests during the manufacturing process

Control tests carried out during antigen production include: virus titration, presence of thiosulphate (to confirm complete neutralisation of the inactivant), residual live virus (inactivation), identity of BTV serotype and bacterial and fungal sterility. Validation of in-process tests is satisfactory. The in-process tests are deemed to be sufficient to control all the critical steps in the manufacturing process.

Control tests on the finished product

The description of the following methods used for the control of the finished product was provided: appearance, volume, identity, *in vivo* potency, identification and quantification of the adjuvant aluminium hydroxide, thiomersal, sterility and pH. Appropriate specifications for each of the tests have been set.

Potential cross-reactions between strains have not been investigated so it is not possible to use a single potency test that is valid for all the strains included in the multi-strain dossier. For this reason, an individual potency test will be carried out for each of the monovalent vaccines that can be formulated within this multi-strain dossier (monovalent BTV-1, monovalent BTV-8 and monovalent BTV-4).

The identification of the active substance in the finished product for each monovalent vaccine is confirmed in the antigen bulk before blending, using the same specific RT-PCR techniques applied to the BTV master and working seeds.

The potency of the finished product is demonstrated for each monovalent vaccine in separate vaccination-challenge tests in transgenic mice where clinical signs are used as endpoints for the evaluation of potency, which is done by comparison with a reference vaccine. Validation of the potency tests for each monovalent vaccine was assessed and accepted during the authorisation of the predecessor product (Zulvac BTV Ovis) and other related centralised procedures. The procedures for

monitoring performance and replacement of reference vaccines and challenge stocks have been adequately described.

As a consequence of the applicant's original proposal to have different antigen content specifications for BTV-8 vaccine intended for use in "sheep only" and for "cattle and sheep", the applicant originally also proposed to use different potency tests for the release of batches of BTV-8 vaccine intended for use in sheep only and batches intended for use in both cattle and sheep. However, it was concluded that the proposal for two BTV-8 formulations for the different species was not acceptable, therefore the applicant agreed to use a single potency test for release of BTV-8 batches.

Overall, sufficient data have been provided to demonstrate that each of the potency tests, as performed, will be able to discriminate between standard and sub-standard batches of vaccine so that only potent batches of vaccines will be released to the market.

The minimum and maximum antigen contents proposed for the monovalent BTV-1 vaccines for sheep and cattle are harmonised ($10^{6.7}$ – $10^{7.4}$ $TCID_{50}$/dose), therefore Zulvac BTV monovalent vaccines against BTV-1 for sheep and cattle will have the same composition. The BTV-1 potency test used in the predecessor authorisation (Zulvac BTV Ovis) for sheep has also been previously accepted for use to control the potency of Zulvac 1 Bovis for cattle, therefore the proposed BTV-1 potency test is relevant for the control of BTV-1 vaccine for use in both sheep and cattle and is satisfactory.

The applicant has confirmed that monovalent BTV-4 vaccine is intended only for use in sheep and will not be used in cattle. Therefore, it is accepted that the addition of cattle to the multi-strain dossier does not impact the antigen content specifications or the potency test for the BTV-4 vaccine.

The in-process and finished product control tests (other than potency) remain the same as agreed during the authorisation of the predecessor product (Zulvac BTV Ovis) and are considered satisfactory.

Batch-to-batch consistency

Adequate data have been provided to support consistent production of monovalent finished product.

Stability

Stability data have been presented for the first virus passages that can be frozen at < -60 °C and used for the initiation of other production runs. The data presented are supportive of the proposed storage period of 24 months at -70 °C ± 10 °C.

Stability data have been presented for the bulk antigens of BTV-1 and BTV-8. The data presented are supportive of the proposed storage period of 12 months at +2 °C to +8 °C.

The stability of the finished product has been demonstrated using batches of monovalent and bivalent BTV-1 and BTV-8 vaccine from the applicant's range of BTV vaccines previously authorised for use in either cattle or sheep. The data presented were supportive of the proposed storage period of 12 months at +2 °C to +8 °C. However, no data are currently available to support either the stability of inactivated BTV-4 antigens or the stability of the monovalent BTV-4 vaccine. Consequently, the same shelf life of 12 months at +2 °C to +8 °C for all the possible monovalent combinations in Zulvac BTV was considered acceptable, pending ongoing stability studies. The efficacy of the antimicrobial preservative was satisfactorily demonstrated.

Overall conclusions on quality

The qualitative and quantitative particulars of the vaccine suspension and the containers are described adequately.

The production process is described in sufficient detail to give confidence that the manufacture will yield a safe and effective vaccine of consistent quality and adequate stability suitable for the expected use of the vaccine in the EU.

The option to produce BTV antigens in bioreactors, which was subject of an existing post-authorisation measure, has been removed from the marketing authorisation dossier. Annex II (D) has been amended accordingly.

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

The in-process controls during manufacture and control tests on the finished product are appropriate to ensure the compliance with the quality specifications mentioned. Acceptance limits are adequately established.

A shelf life of 12 months for all the possible strain monovalent combinations in Zulvac BTV was considered acceptable, pending ongoing stability studies for BTV-4.

Overall, it is considered that the presented analytical dossier is adequate and sufficiently detailed to give confidence that the finished product is produced according to a consistent procedure of adequate standards and including adequate controls.

Part 3 – Safety

Introduction and general requirements

Zulvac BTV is a multi-strain dossier for an inactivated and adjuvanted vaccine against BTV containing a maximum of one of three BTV serotypes (BTV-1, BTV-4 and BTV-8) intended for use in sheep and a maximum of one of two BTV serotypes (BTV-1 and BTV-8) intended for use in cattle. A sufficient amount of each of the corresponding BTV strain is contained in the vaccine so the relative potency for each strain in the finished product is greater than or equal to one (RP≥1) for BTV-1 and BTV-8 and greater than or equal to one (RP≥1) at release or to 0.8 (RP≥0.8) at the end of shelf life for BTV-4. For cattle, the proposed antigen content in $TCID_{50}$ per dose according to pre-inactivation titre is between a minimum of $10^{6.7}$ and a maximum of $10^{7.4}$ for monovalent BTV-1 vaccine and between a minimum of $10^{7.3}$ and a maximum of $10^{7.4}$ for monovalent BTV-8. The vaccine is adjuvanted with aluminium hydroxide (Al^{3+} as hydroxide) and Quil A (*Quillaja saponaria* saponin extract) at final concentrations per dose of 4 mg and 0.4 mg, respectively, and contains thiomersal as preservative at final concentration of 0.2 mg per dose.

In cattle, the proposed primary vaccination schedule is two doses of 2 ml dose administered three weeks apart by the intramuscular (IM) route. The minimum age of vaccination is 3 months. The proposed revaccination schedule for protection against BTV serotype 1 is a single 2 ml dose every 12

months and for protection against BTV serotype 8 is two doses of 2 ml administered three weeks apart (i.e. a repeat of the primary vaccination schedule).

The vaccine is indicated for use during pregnancy and lactation in cattle. No data are available on safety in breeding males. According to the summary of product characteristics (SPC), the vaccine should only be used in breeding males according to the benefit-risk assessment by the responsible veterinarian and/or national competent authorities on the current vaccination policies against BTV.

A number of adverse reactions are described in the SPC. Vaccination in cattle may cause transient increase in rectal temperature of up to 2.7 °C during the 48 hours following vaccination and local reactions of up to 5 cm in diameter, which resolve within 25 days after administration of a single dose. Local reactions may increase slightly following the second dose, in this case lasting up to 15 days.

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

The safety of Zulvac BTV was evaluated according to the guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) (EMA/CVMP/IWP/105506/2007).

Most of the safety data included in this extension to the multi-strain dossier to include cattle as an additional target species have already been assessed for the authorised vaccines Zulvac 1 Bovis (EMEA/V/C/002334, centrally authorised on 05/08/2011), Zulvac 8 Bovis (EMEA/V/C/000145, centrally authorised on 15/01/2010) and Zulvac 1+8 Bovis (EMEA/V/C/002251, centrally authorised on 14/03/2011).

Safety documentation

Four safety studies were conducted to investigate the safety of the product in cattle. These included two Good Laboratory Practice (GLP) laboratory studies investigating respectively: the safety of the administration of one and repeated dose, and a two-fold overdose; and two field trials investigating the safety of the administration of one and repeated dose and a two-fold overdose in pregnant cows in each stage of gestation and lactating cows. The vaccine was administered by the intramuscular route, as recommended.

The laboratory studies were carried out in cattle of the minimum age recommended for vaccination using batches of Zulvac 1+8 Bovis containing $10^{7.0}$ $TCID_{50}$ of BTV-1 and $10^{7.3}$ $TCID_{50}$ of BTV-8 per 2 ml dose. Production batches were used in the field trials using batches of Zulvac 1+8 Bovis containing $10^{6.7}$ $TCID_{50}$ of BTV-1 and $10^{7.3}$ $TCID_{50}$ of BTV-8 per 2 ml dose. The field trials were conducted to Good Clinical Practice (GCP).

The vaccine batches used in the safety studies were prepared according to Part 2B of the dossier with the following exceptions:

- The amount of saponin used in the adjuvant formulation of two of the three batches (Zulvac 1+8 Bovis batch Nos E-64 and E15717) used in the safety studies was 1 mg per 2 ml dose instead of 0.4 mg as proposed for Zulvac BTV. This is consistent with the approved formulation of Zulvac 1+8 Bovis, which has a different adjuvant composition (1 mg saponin instead of 0.4 mg per 2 ml dose) compared to the other approved vaccines of the Zulvac BTV range. This can be considered a worst-case scenario for safety assessment of the monovalent cattle vaccines proposed to be covered by the present Zulvac BTV multi-strain dossier.

- For cattle, Zulvac BTV is formulated to a maximum of one strain (BTV-1 or BTV-8) with a proposed maximum amount of antigen of $10^{7.4}$ $TCID_{50}$. The safety tests have been carried out using batches of the bivalent vaccine Zulvac 1+8 Bovis, which were manufactured with more than the maximum number of strains (two versus one) proposed for the final product of Zulvac BTV. Batch E-64 contained $10^{7.0}$ $TCID_{50}$/dose of BTV-1 and $10^{7.3}$ $TCID_{50}$/dose of BTV-8, which resulted in a combined pre-inactivation titre of $10^{7.5}$ $TCID_{50}$/dose. Batches E15717 and FT010083 contained $10^{6.7}$ $TCID_{50}$/dose of BTV-1 and $10^{7.3}$ $TCID_{50}$/dose of BTV-8, which resulted in a combined pre-inactivation titre of $10^{7.4}$ $TCID_{50}$/dose. The CVMP pragmatically accepted this interpretation of the multi-strain guideline in the original authorisation of Zulvac BTV Ovis and the batches used here are considered to meet the requirements of the multi-strain guideline.

Study no.	***Study title***	***Batch used***	***Antigen content***
Laboratory study			
115-B1-E-05-10	Safety study of the repeated administration of one dose of Zulvac 1+8 Bovis vaccine to calves (three 2 ml doses, each administered three weeks apart)	Zulvac 1+8 Bovis batch E-64	BTV-1: $10^{7.0}$ $TCID_{50}$/2 ml dose BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose
115-B1-E-02-10	Safety study of the administration of an overdose of Zulvac 1+8 Bovis in calves (one 4 ml double dose)	Zulvac 1+8 Bovis batch E-64	BTV-1: $10^{7.0}$ $TCID_{50}$/2 ml dose BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose
Field trial			
115-B2-E-02-11	Safety of the vaccine Zulvac 1+8 Bovis in dairy cows under field conditions (two 2 ml doses administered three weeks apart)	Zulvac 1+8 Bovis batch E15717	BTV-1: $10^{6.7}$ $TCID_{50}$/2 ml dose BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose
115-B2-E-13-09	Safety of the vaccine Zulvac 1+8 Bovis in dairy cows under field conditions (one 4 ml double dose)	Zulvac 1+8 Bovis batch FT010083	BTV-1: $10^{6.7}$ $TCID_{50}$/2 ml dose BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose

Laboratory tests

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

Study 115-B1-E-05-10

Study 115-B1-E-05-10 was performed in order to verify the safety of the administration of repeated administration of a single dose of the vaccine Zulvac 1+8 Bovis in 3-month-old calves. This GLP study was assessed during the original registration procedure of Zulvac 1+8 Bovis (EMEA/V/C/002473). A total of 15 Friesian calves negative for antibodies to BTV-1 and BTV-8 were

included. Of these, 10 calves were administered intramuscularly three times three weeks apart (D0, D21 and D42) with a single dose (2 ml) of Zulvac 1+8 Bovis (Batch E-64 - BTV-1: $10^{7.0}$ $TCID_{50}$/2 ml dose and BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose), while 5 calves were administered phosphate buffered saline (PBS) (2 ml), at the same time points.

Follow-up after administration included blood sampling, clinical observation of animals to detect anaphylactic reactions post vaccination, measurement of rectal temperatures on D-1, D0, D0+4h, and daily during the following four days post vaccinations. Local reactions at the injection site were recorded from D0 to D14 post vaccinations. Daily observations on the general health conditions were made during the same period. Euthanasia and histological examinations of the injection sites were made on calves four weeks after the third vaccination.

Results showed that the calves did not present any general reactions (anaphylactic shock/vomiting), only transitory rectal temperature increases (mean 0.7 ºC and 0.5 ºC after the second and third vaccination, respectively, on days 1 and 2 post injection), and no local reactions. Post mortem histological examinations showed mild to moderate granulomatous myositis in 10-30% of the calves with an average volume less than 0.1 cm^3 (measured 4 weeks post the third administration).

The study was considered suitable to support the multi-strain dossier. Based on the above results, the administration of a single dose and of the repeated administration of a single dose of Zulvac BTV with serotypes 1 and 8 is considered safe. Adverse reactions such as local reactions and transient increase in temperature are adequately addressed in the SPC.

Safety of one administration of an overdose

Study 115-B1-E-02-10

Although it is not currently a requirement for inactivated vaccines, Study 115-B1-E-02-10 was performed in order to verify the safety of the administration of an overdose of the vaccine Zulvac 1+8 Bovis in 3-month-old calves. This GLP study was assessed during the original registration procedure of Zulvac 1+8 Bovis (EMEA/V/C/002473). A total of 15 Friesian calves negative for antibodies to BTV-1 were included. Of these, 10 calves were administered a double dose (4 ml) of Zulvac 1+8 Bovis from batch E-64 (BTV-1: $10^{7.0}$ $TCID_{50}$/2 ml dose and BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose), while 5 calves were administered PBS (4 ml).

Follow-up after administration included blood sampling, clinical observation of animals to detect anaphylactic reactions during the hours after vaccination, measurement of rectal temperatures on D-1, D0, D0+4h, and daily during the following four days. Local reactions at the injection site were recorded from D0 to D14 after vaccination or until total disappearance of the reactions. Daily observations on the general health conditions were made during the same period.

Results showed that the calves did not present any general reactions (anaphylactic shock/vomiting). The overdose of the vaccine Zulvac 1+8 Bovis induced a slight and transient significant mean rectal increase of 1.3 ºC in the vaccinated calves 24 hours after inoculation. On day 2 after vaccination rectal temperatures had normalised.

The study was considered suitable to support the multi-strain dossier. Based on the above results, the administration of an overdose of Zulvac BTV with serotypes 1 and 8 is considered safe. Adverse reactions such as local reactions and transient increase in temperature are adequately addressed in the SPC.

Examination of reproductive performance

The safety of the vaccine in pregnant cattle has not been investigated in laboratory studies. For cattle, the examination of reproductive performance was investigated in two field trials. Annex 1 Commission Directive 2009/9/EC allows for studies for examination of reproductive performance to form part of the field safety studies. The results from the field studies were supportive of the safety of the vaccine in pregnant cattle and during lactation. Safety has not been evaluated in breeding males; however, a suitable warning has been included in the SPC to mitigate the risk.

Examination of immunological functions

No further studies were conducted to investigate the effects of Zulvac BTV on immunological functions but no adverse effects were observed in any of the safety or efficacy studies. The vaccine is not expected to affect negatively the immune response of the vaccinated animal as it is an inactivated vaccine for which the adjuvants have been shown to be safe.

Special requirements for live vaccines

Not applicable.

User safety

A user safety risk assessment has been conducted in accordance with the CVMP guideline (EMEA/CVMP/IWP/54533/2006).

Due to the nature and concentration of its active substances (inactivated bluetongue virus – maximum one of the following BTV serotypes: BTV-1, BTV-8 or BTV-4) and other constituents, the vaccine does not pose any specific risk to the user when used as recommended.

The addition of cattle as a target species and the proposed route of administration (IM) are not expected to change the conclusion of the user safety assessment reached at the time of initial marketing authorisation of Zulvac BTV Ovis.

Study of residues

The addition of cattle as a target species and the proposed route of administration (IM) are not expected to change the conclusion of study of residues as agreed at the authorisation procedure for Zulvac BTV Ovis.

Maximum residue limits (MRLs)

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

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

Withdrawal periods

The withdrawal period is set at zero days.

Interactions

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

Field studies

Study 115-B2-E-02-11

This GCP study was conducted to demonstrate the safety of Zulvac 1+8 Bovis in dairy cows (pregnant and lactating) under field conditions. It was included as a specific obligation at time of first authorisation of that vaccine (EMEA/V/C/002473) and assessed by the CVMP at the time of its first annual review (EMEA/V/C/002437/S/0003).

A total of 120 Friesian dairy cows at different physiological phase (not pregnant and lactating and at different stages of gestation) were included. Sixty cows were vaccinated and revaccinated 3 weeks apart with 2 ml of the vaccine (Batch E15717 - BTV-1: $10^{6.7}$ $TCID_{50}$/2 ml dose and BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose, saponin content 1 ml/dose) and 60 control cows (15 from each treatment group) were administered 2 ml of placebo (PBS). The examined safety reactions included adverse reactions (local and general), rectal temperatures, milk production and reproductive parameters (return to oestrus, confirmed gestations, abortions).

The results of the study demonstrated that the administration of this vaccine according to the SPC was safe when used under field conditions. Although statistically significant higher rectal temperatures were observed in vaccinated cows 24 hours after both the first and second administration, only one single animal at each time point showed a temperature increase above 2 °C. Slight to moderate local reactions at the injection site were recorded. The vaccine did not induce general adverse reactions and did not have any effect in milk production and reproductive performance regardless of the physiological phase and the parity of cows at the time of vaccination. Adverse reactions are adequately addressed in the SPC.

Study 115-B2-E-13-09

Although it is not currently a requirement for inactivated vaccines, this GCP study was conducted to evaluate the safety of the administration of a single overdose (4 ml) of Zulvac 1+8 Bovis to dairy cows (pregnant and lactating) under field conditions. An interim report of this field study was assessed at time of initial authorisation of Zulvac 1 Bovis (EMEA/V/C/002334), where that vaccine was authorised with a specific obligation to complete the study. The final report was provided and assessed during the first annual review of the product, where it was concluded that the specific obligation was fulfilled.

A total of 182 Friesian dairy cows at different physiological phase (not pregnant and lactating, and at different stages of gestation) were included. Ninety-three cows were vaccinated with a double

dose (4 ml) of the vaccine (Batch FT010083 - BTV-1: $10^{6.7}$ $TCID_{50}$/2 ml dose and BTV-8: $10^{7.3}$ $TCID_{50}$/2 ml dose) and 89 control cows were administered 4 ml of placebo (PBS).

Safety follow-up after vaccination included adverse reactions (local and general), rectal temperatures, milk production and reproductive parameters (return to oestrus, confirmed gestations, abortions).

The results of the study showed that an overdose of the vaccine was safe in cattle in the field. No negative effect was demonstrated in pregnant and lactating cows due to vaccination. A transitory rectal temperature increase for up to 2.1 ºC was recorded. This maximum increase occurred in one cow at 1 day after the injection and then rectal temperatures returned to normal values. Slight local reactions (nodules of diameter < 2 cm) appeared at the injection site in 37.5% (9 out of 24) vaccinated animals. Moderate reactions (nodules of diameter up to 5 cm) were observed in 4.2% (1 out of 24) of the animals. Local reactions totally disappeared 57 days after vaccination. The adverse reactions have been duly reflected in the SPC.

The above field studies were considered suitable to support the multi-strain dossier. Based on the above described results, the administration of a single dose, of the repeated administration of a single dose and the administration of an overdose of Zulvac BTV with serotypes 1 and 8 were considered safe in pregnant cows in each stage of gestation and in lactating cows under field conditions. Adverse reactions such as local reactions and transient increase in temperature are adequately addressed in the SPC.

Pharmacovigilance data available for Zulvac 1 Bovis, Zulvac 8 Bovis and Zulvac 1+8 Bovis have been provided, which are supportive of the safety profile observed in the safety studies and indicate that there have not been any changes in the safety profiles of these vaccines based on the data gathered from field use since authorisation.

Environmental risk assessment

An environmental risk assessment has been conducted in accordance with the CVMP Note for Environmental Risk Assessment for Immunological Veterinary Medicinal Products (EMEA/CVMP/074/95). This is the same environmental risk assessment as the one provided for Zulvac BTV Ovis, with editorial revision to include cattle as a target species and the proposed route of administration (IM).

The addition of cattle as a target species and the proposed route of administration (IM) in Zulvac BTV are not expected to change the conclusion of the environmental risk assessment agreed at authorisation of Zulvac BTV Ovis.

Based on the phase I assessment, a study of phase II has not been considered necessary.

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

Overall conclusions on the safety documentation

In accordance with the Guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) (EMA/CVMP/IWP/105506/2007), the safety of Zulvac BTV in the additional target species, cattle, has been demonstrated in a total of four safety laboratory and field studies using batches of vaccine

manufactured with more than the maximum number of strains proposed for the final product (n=1) and with the maximum amount of antigen ($10^{7.4}$ $TCID_{50}$/dose) or higher.

Safety has been demonstrated using the recommended route of administration (intramuscular) of a single dose, of the repeated administration of a single dose and the administration of an overdose in calves of the minimum recommended age at first vaccination in the laboratory studies and in pregnant (each stage of gestation) and lactating cows in the field studies. The results of the safety studies have been adequately reflected in section 4.6 and 4.10 of the SPC.

Reproductive safety was investigated in two different field studies. The product was found to be safe when used in pregnant animals and in lactating animals. The safety of the vaccine has not been investigated in breeding males. Suitable warnings have been included in section 4.7 of the SPC.

Zulvac BTV is a conventional inactivated vaccine containing active substances and excipients with no known adverse effect on immunological functions.

A withdrawal period of zero days has been justified.

No specific studies were carried out to investigate the interactions with other veterinary medicinal products. This is duly reflected in the relevant section of the SPC.

The risk of the vaccine to the end user and the environment is considered to be negligible when used as recommended.

Pharmacovigilance data of the authorised vaccines Zulvac 1 Bovis, Zulvac 8 Bovis and Zulvac 1+8 Bovis are supportive of the safety profile observed in the laboratory studies.

Part 4 – Efficacy

Introduction and general requirements

Zulvac BTV is a multi-strain dossier for an inactivated and adjuvanted vaccine against bluetongue virus containing a maximum of one of three BTV serotypes (BTV-1, BTV-4 and BTV-8) intended for use in sheep and a maximum of one of two BTV serotypes (BTV-1 and BTV-8) intended for use in cattle. The vaccine is currently authorised as Zulvac BTV Ovis for sheep and the current extension application is to add cattle as a new target species. A sufficient amount of each of the corresponding BTV strain is contained in the vaccine so the relative potency for each strain in the finished product is greater than or equal to one (RP≥1) for BTV-1 and BTV-8 and greater than or equal to one (RP≥1) at release or to 0.8 (RP≥0.8) at the end of shelf life for BTV-4.

For cattle, the proposed antigen content in $TCID_{50}$ per dose according to pre-inactivation titre is between a minimum of $10^{6.7}$ and a maximum of $10^{7.4}$ for monovalent BTV-1 vaccine and between a minimum of $10^{7.3}$ and a maximum of $10^{7.4}$ for monovalent BTV-8. The vaccine is adjuvanted with aluminium hydroxide (Al^{3+} as hydroxide) and Quil A at final concentrations per dose of 4 mg and 0.4 mg, respectively, and contains thiomersal as preservative at final concentration of 0.2 mg per dose.

The vaccine is indicated for active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus, serotype 1 or serotype 8. The vaccine is administered intramuscularly to cattle from 12 weeks of age as two 2 ml doses separated by 3 weeks. The claimed onset of immunity (OOI) is 15 days for BTV-1 and 25 days for BTV-8. The claimed duration of immunity (DOI) is 12 months for serotype 1 and serotype 8. A revaccination schedule of a 2 ml dose

every 12 months has been recommended for protection against BTV-1 and of two 2 ml doses separated by 3 weeks every 12 months for protection against BTV-8.

The vaccine is stated to be suitable for use during pregnancy and lactation. The safety and the efficacy of the vaccine have not been established in breeding males. The SPC states that in this category of animals the vaccine should be used only according to the benefit-risk assessment by the responsible veterinarian and/or national competent authorities on the current vaccination policies against BTV.

No information is available on the use of the vaccine in seropositive animals including those with maternally derived antibodies.

The efficacy of Zulvac BTV was evaluated according to the CVMP Guideline on data requirements for multi-strain dossiers for inactivated vaccines against avian influenza (AI), Bluetongue (BT) and Foot-and-Mouth disease (FMD) (EMA/CVMP/IWP/105506/2007).

The multi-strain dossier from Zulvac BTV has been developed by combining data of three vaccines already authorised in Europe: Zulvac 1 Bovis (EMEA/V/C/002334), centrally authorised on 05/08/2011; Zulvac 8 Bovis (EMEA/V/C/000145), centrally authorised on 15/01/2010; and Zulvac 1+8 Bovis (EMEA/V/C/002251), centrally authorised on 14/03/2011.

Challenge model

The BTV-1 and BTV-8 challenge strains used in the efficacy studies in cattle were homologous to the respective vaccine strains and considered justified by the applicant that, at the time the experiments were conducted and due to the emergency nature of the studies, there was no time to source and qualify suitable heterologous strains. Based on the sequence and epidemiological information provided, the relevance of the homologous BTV-1 and BTV-8 challenge strains to the respective current epidemiological situation of BTV-1 and BTV-8 in the EU is justified. The strains originated from infected sheep and were administered by the intravenous route to animals as a single dose containing 2 ml. The aim of the challenge model was that all control animals become viraemic after challenge and the viraemia was detectable by RT-qPCR. A summary of six challenge studies performed with BTV-1 and nine with BTV-8 shows that, respectively, 98% and 99% of control calves became viraemic post-challenge.

Efficacy parameters and tests

The efficacy parameters as chosen by the applicant, investigated in the efficacy studies, included detection of BTV genome in blood samples by RT-qPCR (viraemia) and detection of neutralising antibodies against different BTV serotypes by a seroneutralisation test. The parameters chosen are considered appropriate for evaluating the efficacy of the product. Validation of the tests has been provided, demonstrating their suitability.

Efficacy documentation

Seven laboratory studies were submitted to investigate the efficacy of the product. Laboratory studies were well documented and carried out in calves of the minimum age recommended for vaccination and vaccinated by the recommended schedule and route of administration. The mono-strain batches of vaccines used in the studies were manufactured according to Part 2 of the dossier

and pivotal studies were carried out using batches containing the proposed minimum amount of antigen in each of the proposed mono-strain vaccines.

No field trials have been conducted, which has been justified on the basis of experience and data gathered so far with the respective monovalent and bivalent BTV vaccines that have been marketed in the EU in the recent years.

Study no.	**Study type**	**Vaccine**	**Batch used and antigen content**
115-B1-E-01-07	OOI	Zulvac 1 Bovis	Vaccine 1 [batch number BTV1-A (190707)]: $10^{6.93}$ $TCID_{50}$/dose Vaccine 2 (dilution of vaccine 1 to 50%): $10^{6.63}$ $TCID_{50}$/dose
115-B1-E-01-10	OOI	Zulvac 1 Bovis	Batch E-58: $10^{6.7}$ $TCID_{50}$/dose Batch E-58 (dilution of vaccine to 50%): $10^{6.4}$ $TCID_{50}$/dose
115-B1-E-11-07	OOI	Zulvac 8 Bovis	Vaccine A [batch number BTV-8 vaccine A (231107)]: $10^{7.3}$ $TCID_{50}$/dose Vaccine B [batch number BTV-8 vaccine B (231107)]: $10^{7.0}$ $TCID_{50}$/dose Vaccine C [batch number BTV-8 vaccine C (231107)]: $10^{6.7}$ $TCID_{50}$/dose Vaccine D[batch number BTV-8 vaccine D (231107)] : $10^{6.4}$ $TCID_{50}$/dose
115-B1-E-19-08	OOI	Zulvac 8 Bovis	Batch E-11: $10^{7.3}$ TCI_{D50}/dose Batch E-12: $10^{7.0}$ $TCID_{50}$/dose Batch E-13: $10^{6.7}$ $TCID_{50}$/dose Batch E-14: $10^{6.4}$ $TCID_{50}$/dose
115-B1-E-03-08	DOI	Zulvac 1 Bovis	Batch E-10: $10^{6.7}$ $TCID_{50}$/dose
	Revaccination	Zulvac 1 Bovis	Batch E-36: $10^{6.7}$ $TCID_{50}$/dose
115-B1-E-09-07	Revaccination	Zulvac 1+4 Bovis Zulvac 1+4 Bovis Zulvac 1+4	Batch BTV 1+4 Pre-immunovaccine A: $10^{7.0}$ $TCID_{50}$/dose BTV-1; $10^{7.3}$ $TCID_{50}$/dose BTV-4 Batch BTV 1+4 Pre-immunovaccine B: $10^{6.7}$ $TCID_{50}$/dose BTV-1; $10^{7.0}$ $TCID_{50}$/dose BTV-4 Batch BTV 1+4 Pre-immunovaccine C: $10^{6.4}$ $TCID_{50}$/dose BTV-1; $10^{6.7}$ $TCID_{50}$/dose BTV-4

		Bovis Zulvac 1 Bovis Zulvac 4 Bovis Zulvac 1 Bovis	Batch BTV 1 Pre-immunovaccine B: $10^{6.7}$ $TCID_{50}$/dose BTV-1 Batch E-6: $10^{7.0}$ $TCID_{50}$/dose BTV-4 Batch E-36: $10^{6.7}$ $TCID_{50}$/dose BTV-1
115-B1-E-26-08	DOI	Zulvac 8 Bovis	Batch E-23: $10^{7.3}$ $TCID_{50}$/dose Batch E-24: $10^{7.0}$ $TCID_{50}$/dose Batch E-25: $10^{6.7}$ $TCID_{50}$/dose

Laboratory trials

Dose determination

The proposed minimum and maximum concentration of each antigen per dose have been established based on the safety and efficacy studies included in Part 3 and Part 4 of the multi-strain dossier.

For each vaccinal strain, BTV-1 and BTV-8, two minimum immunogenic dose studies have been performed (see below in section Onset of Immunity).

Onset of immunity (OOI)

Four studies were carried out in cattle from 2.5 months of age to investigate the onset of protection by the recommended administration route.

Study 115-B1-E-01-07 (BTV-1)

This study aimed to evaluate the efficacy of two different antigen concentrations in Zulvac 1 Bovis vaccine in order to establish the lowest vaccine concentration able to prevent viraemia (presence of viral genome in blood) in vaccinated calves. This study was assessed by the CVMP during the original application procedure for Zulvac 1 Bovis (EMEA/V/C/002334).

A total of 17 Friesian calves, 2.5 months of age and tested negative for antibodies to BTV-1 by ELISA, were included in the study. Three groups of calves were made (Group 1. n=5; Group 2. n=5, Group 3. n=7). Calves in Groups 1 and 2 were vaccinated and revaccinated after three weeks with two different BTV-1 vaccines (Vaccine 1: $10^{6.93}$ $TCID_{50}$ of BTV-1 per 2 ml dose; Vaccine 2: $10^{6.63}$ $TCID_{50}$ of BTV-1 per 2 ml dose). The challenge load was 2 ml of BTV-1 challenge virus ($10^{6.55}$ $TCID_{50}$/ml) via intravenous route per animal.

The calves were monitored after each injection for the appearance of any systemic reactions associated with the administration of vaccine (anaphylactic shock, anorexia, etc.). Rectal temperature was measured before each injection, at D0+4h and daily during the next two days.

Injection site reactions were observed post vaccination for 14 days after each injection, blood samples were taken on D0 and D35 (the day prior to challenge) and serological responses measured by ELISA and seroneutralisation (SN). After challenge, blood samples were extracted from the animals on Days 3, 5, 7, 8, 11, 14, 18, 21, 25 and 28 post challenge, for the evaluation of presence of the BTV genome by real-time RT-qPCR. The animals were monitored daily during 15 days post challenge for the appearance of clinical signs associated with the disease (rectal temperature, nasal and/or ocular discharge, coughing, dyspnoea, limping, and prostration).

Results showed that viraemia was prevented in both vaccinated groups (viral genome had not been detected by real-time RT-qPCR technique at 28 days post challenge). The two tested vaccines induced a significant post-vaccination serological response (antibody titres detected by ELISA and SN) in the animals. Increase in rectal temperatures was not statistically significant in vaccinated animals and none of the calves manifested local reactions at the injection site. OOI was therefore supported to start from 15 days after completion of the initial vaccination scheme in this homologous challenge model.

This pivotal study supports the proposed claim for Zulvac BTV for active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus serotype 1 and OOI of 15 days. The minimum efficacious dose for BTV-1 mono-strain vaccine in cattle is set at $10^{6.7}$ $TCID_{50}$/ 2 ml dose.

Study 115-B1-E-01-10 (BTV-1)

This study aimed to evaluate the efficacy of Zulvac 1 Bovis batch E-58, in order to test if the vaccine was able to prevent viraemia in 100% of the vaccinated and challenged calves. This study was assessed by the CVMP during the original application procedure for Zulvac 1 Bovis (EMEA/V/C/002334).

A total of 30 Friesian calves, 2.5 months of age and tested negative for antibodies to BTV-1 by ELISA were included in the study. Three groups of calves were made (Group 1. n=12; Group 2. n=12, Group 3. n=6). Calves in Groups 1 and 2 were vaccinated and revaccinated after three weeks with two different BTV-1 vaccines (Vaccine 1: $10^{6.7}$ $TCID_{50}$ of BTV-1 per 2 ml dose; Vaccine 2: $10^{6.4}$ $TCID_{50}$ of BTV-1). Twenty-one days after revaccination (D42), 10 calves from Group 1, 10 calves from Group 2 and 5 calves from Group 3 were challenged with BTV-1. Challenge load: 2 ml of challenge virus ($10^{6.55}$ $TCID_{50}$/ml) via intravenous route per animal.

The calves were monitored after each injection for the appearance of any systemic reactions associated with the administration of vaccine (anaphylactic shock, anorexia, etc.). Blood samples were taken on D0 and D42 (prior to challenge) and serological responses measured by ELISA and SN. After challenge, blood samples were taken from the animals on Days 0, 3, 5, 7, 10, 13, 17, 20, 24 and 27 post infection, for the evaluation of the presence of the BTV genome with the real-time RT-qPCR technique. The animals were monitored on Days 0, 3, 5, 7, 10, 13, 17, 20, 24 and 27 post infection for the appearance of clinical signs associated with the disease (rectal temperature, nasal and/or ocular discharge, nasal and/or ocular oedema, lameness and prostration).

Results showed that none of the calves showed any systemic reactions neither after the first nor the second injection. Serological response after vaccination was detected both as ELISA and SN titres. Rectal temperatures were significantly increased in controls compared to vaccinates on Day 7 post challenge (day of maximal viraemia). However, the rectal temperature increase in the controls was very slight. Clinical signs after BTV-1 challenge were observed in 2 vaccinated calves, but the

symptoms were rather mild. Viral genome was not detected in any of the vaccinated calves in Groups 1 and 2 during 27 days after challenge with BTV serotype 1, whereas in all unvaccinated calves challenged (Group 3), the viral genome was detected from Day 3 after challenge.

The study was considered supportive only of the proposed claim for Zulvac BTV for active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus serotype 1 as the OOI demonstrated in the study was 21 days after completion of the primary vaccination schedule, while that proposed for Zulvac BTV is 15 days. The minimum efficacious dose for BTV-1 mono-strain vaccine in cattle was set at $10^{6.7}$ $TCID_{50}$/ 2 ml dose.

Study 115-B1-E-11-07 (BTV-8)

This study aimed to evaluate the efficacy of four experimental Zulvac 8 Bovis vaccines formulated with different amounts of BTV-8 antigen, and standard amounts of adjuvants. This study was assessed by the CVMP during the original application procedure for Zulvac 8 Bovis (EMEA/V/C/000145).

The vaccine was administered as two doses of 2 ml, administered 3 weeks apart, in calves of minimum age. The efficacy of the vaccines was evaluated based on their capacity to reduce or prevent viraemia after a BTV-8 challenge carried out 25 days after the second vaccination.

Healthy calves, 2.5-months-old and seronegative against BTV were randomly distributed in 5 groups as follows: 4 groups (1 to 4) of calves vaccinated and revaccinated with the four experimental Zulvac 8 Bovis vaccine preparations (formulated to contain, respectively, Vaccine A: $10^{7.3}$ $TCID_{50}$/dose; Vaccine B: $10^{7.0}$ $TCID_{50}$/dose; Vaccine C: $10^{6.7}$ $TCID_{50}$/dose; Vaccine D: $10^{6.4}$ $TCID_{50}$/dose) and one group (5) of controls. On D0, each calf of Groups 1, 2, 3 and 4 was intramuscularly (IM) injected with a 2 ml dose of the corresponding vaccine. About 3 weeks later (D19), these animals were revaccinated under the same conditions. Calves allocated in Group 5 acted as control for the challenge experiment and remained untreated.

Blood samples were collected before the 1st vaccination (D0), approximately 2 weeks after 1st vaccination (D16); about 2 and 3 weeks after revaccination (D32 and D40) and on D44 before challenge, in order to detect SN and ELISA antibodies against BTV-8.

A challenge with a virulent BTV-8 field isolate was carried out 25 days after the 2nd vaccination (D44). From the day of challenge, daily starting 3 days after and up to D14, on D17, D19 and D24 after challenge, animals were monitored for the appearance of major clinical signs reported to being observed during BTV-8 infection (these signs including increase of rectal temperature, nasal discharge, watering, coughing, dyspnoea, limping, prostration and mortality). Blood samples were collected on the day of challenge (D44) and then 3, 5, 7, 10, 13, 17, 20, 24 and 27 days after. The definition of protection was the consistent absence of viral load detectable by real-time RT-qPCR in all the vaccinated animals during the monitoring period of 4 weeks, defining viral load detectable by real-time RT-qPCR as the one that provides as a result a Ct value lower than 36.0.

Viral genome was not detected in any of the calves vaccinated with the experimental vaccine preparation containing the highest amount of BTV-8 antigen (100% prevention of viraemia). Viral genome was detected in 100% of the control unvaccinated calves from 5-7 days post infection.

Based on the results of this study, the administration of two doses of the experimental vaccine Zulvac 8 Bovis formulated at $10^{7.3}$ $TCID_{50}$ BTV-8/dose resulted in a total protection of calves, vaccinated at a minimum age, against viraemia.

This pivotal study supports the proposed claim for Zulvac BTV for active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus serotype 8 and OOI of 25 days. The minimum efficacious dose for BTV-8 mono-strain vaccine in cattle was set at $10^{7.3}$ $TCID_{50}$/2 ml dose.

Study 115-B1-E-19-08 (BTV-8)

This study aimed to evaluate the efficacy of four experimental Zulvac 8 Bovis vaccines formulated with different amounts of BTV-8 antigen, and standard amounts of adjuvants. This study was assessed by the CVMP during the original application procedure for Zulvac 8 Bovis (EMEA/V/C/000145).

This study was designed to compare and support the results of the previous study where there was not a good correlation between the different vaccine concentrations tested and the real-time RT-qPCR results. The vaccine was administered as two doses of 2 ml, 3 weeks apart, in calves of minimum age. The efficacy of the vaccines was evaluated based on their capacity to reduce or prevent viraemia after a BTV-8 challenge carried out 25 days after the second vaccination.

Healthy calves, 2.5-months-old and seronegative against BTV, were randomly distributed in 5 groups as follows: 4 groups (1 to 4) of calves vaccinated and revaccinated with the four experimental Zulvac 8 Bovis vaccine preparations (respectively Batch E-11: $10^{7.3}$ $TCID_{50}$/dose; Batch E-12: $10^{7.0}$ $TCID_{50}$/dose; Batch E-13: $10^{6.7}$ $TCID_{50}$/dose; Batch E-14: $10^{6.4}$ $TCID_{50}$/dose) and 1 group (5) of controls.

On D0, each calf of Groups 1, 2, 3 and 4 was intramuscularly injected with a 2 ml dose of the corresponding vaccine. About 3 weeks later (D21), these animals were revaccinated under the same conditions. Calves allocated in Group 5 acted as control for the challenge experiment and remained untreated.

A challenge with a virulent BTV-8 field isolate was carried out 25 days after 2nd vaccination (e.g. D46). Two ml of the virulent challenge material were inoculated in the jugular vein of each animal. Blood samples were collected on the day of challenge (D46) and then 3, 6, 8, 10, 13, 16, 22, 24 and 27 days after. The definition of protection was the consistent absence of viral load detectable by real-time RT-qPCR in all the vaccinated animals during the monitoring period of 4 weeks, defining viral load detectable by real-time RT-qPCR as the one that provides as a result a Ct value lower than 36.0.

There was a good correlation between the amount of BTV-8 antigen contained in the four experimental vaccine preparations tested and the detection of viral genome (from 100% to 25%). Viral genome was detected in 100% of the control unvaccinated calves from 3-6 days post infection.

The study demonstrated a good correlation between the different vaccine concentrations tested and the real-time RT-qPCR results, and a significant protection of the vaccinated animals. The administration of the experimental vaccine Zulvac 8 Bovis formulated at $10^{7.3}$ $TCID_{50}$ BTV-8/dose resulted in a total protection of calves, vaccinated at a minimum age, against viraemia.

This pivotal study supports the proposed claim for Zulvac BTV for active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus serotype 8 and OOI of 25 days. The minimum efficacious dose was set at $10^{7.3}$ $TCID_{50}$ BTV-8/dose.

Duration of immunity (DOI)

Two different studies were presented to support the DOI after vaccination.

Study 115-B1-E-03-08 (BTV-1)

This study aimed to evaluate if the Zulvac 1 Bovis vaccine was able to prevent viraemia in calves vaccinated and challenged 1 year post vaccination. This study was assessed by the CVMP during the original application procedure for Zulvac 1 Bovis (EMEA/V/C/002334).

A total of 44 Friesian calves, 2.5 to 4 months of age and negative for antibodies to bluetongue virus were included. Batch E-10, containing $10^{6.7}$ $TCID_{50}$/2 ml dose of BTV-1 was used. Two groups of calves were made (n=22 calves were vaccinated and revaccinated after 3 weeks; 22 calves served as non-vaccinated controls). Sixteen calves were challenged with BTV-1 at 15-16 months of age. Challenge load: 2 ml of challenge virus ($10^{6.55}$ $TCID_{50}$/ml) via intravenous route per animal.

After each vaccination, the calves were monitored for the appearance of any systemic reaction associated with the vaccine administration (anaphylactic shock, anorexia, vomiting, etc.). Blood samples were taken from the calves: At D<0 (before 1st vaccination), D18 (before 2nd vaccination), D35, D42, D88, D113, D170, D192/198, D227, D249, D297 and D331. On day D375 the calves were challenged with virulent BTV-1. Blood samples were taken from the animals on Days 0, 3, 5, 7, 10, 13, 17, 20, 24 and 27 post challenge. The animals were also monitored for clinical signs associated with the disease (rectal temperature, nasal and/or ocular discharge, nasal and/or ocular oedema, other oedemas, lameness and prostration).

Results showed that none of the calves manifested any systemic reactions after 1st and 2nd vaccinations. In none of the vaccinated calves (Group 1) was viral genome detected after challenge, whereas in all the 8 non-vaccinated (Group 2) and challenged calves, the viral genome was detected from D5 post inoculation. Clinical infections induced mild and non-specific clinical signs; however on day 7 post challenge control animals presented a higher rectal temperature than the vaccinated ones.

This pivotal study supports the proposed claim for Zulvac BTV for the active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus serotype 1 with DOI of 12 months after completion of the primary vaccination scheme.

Study 115-B1-26-08 (BTV-8)

This study aimed to evaluate if the administration of Zulvac 8 Bovis vaccine is able to prevent viraemia in cattle challenged 7 and 12 months post vaccination. Interim data from this study (7 months DOI) were assessed by the CVMP during the original application procedure for Zulvac 8 Bovis (EMEA/V/C/000145) and the final report was assessed as a post-approval commitment during the subsequent Type II variation procedure EMEA/V/C/145/II/002.

A total of 79 Friesian calves, 2.5 to 4 months of age and negative for antibodies to bluetongue virus were included in the study. Twenty-two calves were vaccinated intramuscularly according to the schedule, with a batch of vaccine containing $10^{7.3}$ $TCID_{50}$ of BTV-8/dose (Group 1), 21 calves with a batch of vaccine containing $10^{7.0}$ $TCID_{50}$ of BTV-8/dose (Group 2), 21 calves with a batch of vaccine containing $10^{6.7}$ $TCID_{50}$ of BTV-8/dose (Group 3) and 15 calves were kept as unvaccinated controls (Group 4). After vaccinations, the calves were monitored for the appearance of any systemic reaction associated with the vaccine administration (e.g. anaphylactic shock, anorexia).

Approximately 7 months (Day 213) after completion of the primary vaccination scheme, seven calves from each of Groups 1 and 2 and four calves from Group 4 were challenged with a virulent BTV-8 strain. Approximately 12 months after completion of the primary vaccination scheme, eleven calves from Group 1 and seven calves from Group 4 were challenged with a virulent BTV-8 strain. The challenged animals were monitored for 27-28 days for appearance of clinical signs and presence of viraemia (by real-time RT-qPCR).

The results of the study showed that none of the calves manifested any systemic reactions after vaccination. No statistically significant differences in clinical signs were observed between vaccinated and control animals post challenge. The results demonstrated that administration of Zulvac 8 Bovis vaccine at a concentration of $10^{7.3}$ $TCID_{50}$/2 ml was capable of preventing viraemia in vaccinated animals up to 12 months after primary vaccination, as viraemia was not detected in any of the vaccinated animals after challenge at 7 months and in only one of the vaccinated animals for one day (Day 5 post challenge) after challenge at 12 months, where it was concluded that this animal was considered non-viraemic as viraemia was not persistent (it is unusual to find one animal viraemic for only one day). In contrast, all the unvaccinated control animals developed viraemia after challenge. DOI of 12 months after completion of the primary vaccination scheme was concluded for Zulvac 8 Bovis.

This pivotal study supports the proposed claim for Zulvac BTV for active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by bluetongue virus serotype 8 and DOI of 12 months after completion of the primary vaccination scheme.

Additional Studies:

Additional serology data from the original BTV-1 DOI study (115-B1-E-03-08) show that revaccination of animals with a single dose of Zulvac 1 Bovis ($10^{6.7}$ $TCID_{50}$/dose) approximately one year after completion of primary vaccination boosted neutralising antibody titres to levels significantly higher than those observed after primary vaccination (e.g. geometric mean titre [GMT] of 139.6 seven weeks after revaccination compared to GMT of 19.0 two weeks after primary vaccination). It was also shown in this study that seroneutralising antibody titres (GMT 29.9) for up to 21 months after primary vaccination with Zulvac 1 Bovis ($10^{6.7}$ $TCID_{50}$/dose) are maintained and are comparable to those at 12 months after primary vaccination (GMT 19.9), where prevention of viraemia was demonstrated in animals in the same group after challenge with a virulent strain of BTV-1.

Meta-analysis of serological results from laboratory efficacy studies conducted with different Zulvac vaccines (including Zulvac 1 Bovis, Zulvac 8 Bovis and Zulvac 1+8 Bovis) has also been provided, which shows that protective immunity against BTV is strongly associated with the presence of neutralising antibodies.

Further data to support a single annual revaccination scheme are provided from a second study (115-B1-E-09-07), which was a comparative study to assess long term serological responses in calves vaccinated with different Zulvac vaccine formulations and doses (Zulvac 1+4 Bovis, Zulvac 1 Bovis and Zulvac 4 Bovis).

A total of 42 Holstein Friesian calves, 2.5 months of age and negative for antibodies to bluetongue virus were randomly allocated into six treatment groups. Group 1 animals were vaccinated according to the schedule with a batch of Zulvac 1+4 Bovis vaccine containing $10^{7.0}$ $TCID_{50}$ BTV-1 and $10^{7.3}$ $TCID_{50}$ BTV-4; Group 2 with Zulvac 1+4 Bovis vaccine containing $10^{6.7}$ $TCID_{50}$ BTV-1 and

$10^{7.0}$ $TCID_{50}$ BTV-4; Group 3 with Zulvac 1+4 Bovis vaccine containing $10^{6.4}$ $TCID_{50}$ BTV-1 and $10^{6.7}$ $TCID_{50}$ BTV-4; Group 4 with Zulvac 1 Bovis containing $10^{6.7}$ $TCID_{50}$ BTV-1; Group 5 with Zulvac BTV 4 Bovis containing $10^{7.0}$ $TCID_{50}$ BTV-4; and Group 6 were unvaccinated controls. In the first part of the study calves in each group were monitored for neutralising antibodies against BTV-1 for up to 10 months after primary vaccination (D317). In the second part of the study, 4 calves from Group 2 and 4 calves from Group 4 were revaccinated with a single dose of Zulvac 1 Bovis containing $10^{6.7}$ $TCID_{50}$ BTV-1 and monitored for neutralising antibodies against BTV-1 on the day of revaccination (D514) and at 47 (D561) and 60 (D574) days post revaccination.

The results of the first part of the study showed that neutralising antibody titres against BTV-1 were detectable approximately 10 months after primary vaccination in all vaccinated groups (GMT ranges from 8 to 32). The results of the second part of the study showed revaccination of animals with a single dose of Zulvac 1 Bovis ($10^{6.7}$ $TCID_{50}$/dose) 16 months after completion of primary vaccination boosted neutralising antibody titres from GMT of 3.4 and 9.0 in Group 2 and 4, respectively, at Day 514 to GMT of 13.5 and 49.3 at 47 and 60 days post revaccination in Group 2 and GMT of 57.0 and 60.4 at 47 and 60 days post revaccination in Group 4. For both groups the neutralising antibody titres against BTV-1 shortly after the single dose booster administered 16 months after vaccination were higher than the ones observed shortly after the primary vaccination scheme.

In conclusion for BTV-1, DOI of 12 months after completion of the primary vaccination scheme in cattle is supported with a revaccination schedule of one dose of vaccine administered every 12 months. There is supplementary evidence of BTV-1 seroneutralising antibody titres indicative of protection for up to 21 months after primary vaccination. For BTV-8, DOI of 12 months after completion of the primary vaccination scheme is supported with an annual revaccination schedule of two doses of vaccine administered three weeks apart.

Maternally derived antibodies (MDA)

The influence of MDA on the efficacy of the vaccine has not been investigated, which is considered acceptable. A standard warning has been included in section 4.4 of the SPC.

Interactions

No information is available of the efficacy of the vaccine when used with any other veterinary medicinal product. A standard warning has been included in section 4.8 of the SPC.

Field trials

No data on field trials have been provided. The absence of data from field studies has been justified on the basis of experience and data gathered so far with the respective monovalent and bivalent BTV vaccines that have been marketed in the EU in recent years. The efficacy profile of these inactivated vaccines is considered satisfactory under field conditions.

Pharmacovigilance data available for Zulvac 1 Bovis, Zulvac 8 Bovis and Zulvac 1+8 Bovis have been provided, which show that no suspected lack of expected efficacy reports (SLEEs) were received since authorisation.

Overall conclusion on efficacy

The efficacy of Zulvac BTV has been demonstrated under laboratory conditions using a vaccination-challenge model developed in the target species where cattle were vaccinated according to the recommended vaccination schedule and challenged with virulent BTV of the corresponding serotype after a defined period of time. The primary variable to determine efficacy was the detection of genome of BTV in blood samples collected after challenge, using a validated RT-qPCR. Absence of viraemia was defined as below the level of detection <3.4 $\log_{10}$ genome copies/ml. Detection of neutralising antibodies against different BTV serotypes by a seroneutralisation test was used as a correlate of protection to support the revaccination claim.

The OOI and DOI studies have been carried out including animals of the minimum recommended age of the target species (12 weeks) and using batches of mono-strain vaccine containing the proposed minimum concentration of the respective antigen per dose ($10^{6.7}$ $TCID_{50}$/dose for the mono-strain vaccine against BTV-1 and $10^{7.3}$ for the mono-strain vaccine against BTV-8).

An OOI of 15 days for BTV-1 and 25 days for BTV-8 after completion of the primary vaccination schedule has been satisfactorily demonstrated.

DOI of 12 months has been demonstrated for the BTV-1 mono-strain vaccine, with a revaccination schedule of one dose of vaccine administered every 12 months. There is supplementary evidence of BTV-1 seroneutralising antibody titres indicative of protection for up to 21 months after primary vaccination. DOI of 12 months has been demonstrated for the BTV-8 mono-strain vaccine, with an annual revaccination schedule of two doses of vaccine administered three weeks apart.

The influence of maternally derived antibodies on the efficacy of the vaccine has not been investigated, which is considered acceptable. A standard warning has been included in section 4.4 of the SPC.

No field data have been generated to supplement the results obtained in the efficacy laboratory studies. This has been justified based on the experience gathered so far with the respective monovalent and bivalent BTV vaccines that have been marketed in the EU in recent years. Pharmacovigilance data available for Zulvac 1 Bovis, Zulvac 8 Bovis and Zulvac 1+8 Bovis have been provided, which show that no suspected lack of expected efficacy reports (SLEEs) were received since authorisation. The justification was considered acceptable.

Part 5 – Benefit-risk assessment

Introduction

Zulvac BTV is an inactivated, bluetongue vaccine consisting of either BTV-1, BTV-4 or BTV-8. It is intended as a multi-strain product, which allows mixing of appropriate strains in response to field need, however in this instance only monovalent vaccines will be produced.

This application is a line extension to add cattle as a new target species to the previously authorised Zulvac BTV Ovis. The strains included are relevant to the current epidemiological situation of BTV in the EU.

The vaccine is already authorised for use in sheep. The proposed new claim is for the active immunisation of cattle from 12 weeks of age for the prevention of viraemia caused by BTV serotype 1 or serotype 8.

The proposed vaccination schedule consists of 2 injections of one dose, with a 3 week interval. A single dose (2 ml) annual re-vaccination scheme with monovalent vaccine BTV-1 is recommended for protection against BTV serotype 1, and in the case of monovalent vaccine BTV-8 for protection against BTV serotype 8 an annual repeat of the primary vaccination schedule is recommended.

The extension application has been submitted in accordance with Article 19 of Commission Regulation (EC) 1234/2008 and Annex I thereof (extensions).

Benefit assessment

Direct therapeutic benefit

The benefit of Zulvac BTV is its efficacy to induce active immunisation in cattle from 12 weeks of age for the prevention of viraemia caused by BTV, serotypes 1 or 8. Efficacy was shown in a number of laboratory studies.

The OOI is 15 days for BTV-1 and 25 days for BTV-8. The DOI is 12 months for BTV-1 and 12 months for BTV-8.

Additional benefits

Even if only monovalent vaccines are allowed, it is expected that the multi-strain approach will contribute positively to availability of BTV-1 and BTV-8 vaccines through adaptability and potential benefits of a rationalised process.

Risk assessment

Quality:

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

Safety:

Measures to manage the risks identified below are included in the risk management section.

Risks for the target animal:

The product is generally well tolerated in cattle and in pregnant and non-pregnant lactating cows, using the recommended route of administration of the vaccine (intramuscular).

Vaccination may cause a transient increase in rectal temperatures and local reactions at the injection site. Local reactions are more common after the repeated administration of a single dose.

Safety in both target species has been adequately demonstrated and any appropriate warnings included on the SPC.

Risk for the user:

The potential risks to the person administering the products as well as other persons in direct contact with the animals have been evaluated in relation with the components of Zulvac BTV. No risks were identified and therefore no specific warnings for users were proposed.

Risk for the environment:

Zulvac BTV 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:

A withdrawal period of zero days was set.

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

Evaluation of the benefit-risk balance

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

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application to add a new target species (cattle) is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No. 726/2004 in conjunction with Directive 2001/82/EC).

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