September 2011
EMA/457946/2011
Veterinary Medicines and Product Data Management

Scientific discussion

1. Summary of the dossier

ZULVAC 1 Ovis is a conventionally produced, liquid and ready-to-use vaccine, and aluminium hydroxide/saponin adjuvanted. The active substance of ZULVAC 1 Ovis is inactivated Bluetongue virus (BTV), serotype 1 an immunological veterinary medicinal product (ATCvet Code QI04AA02) which acts by the development of active immunisation of sheep and preventing viraemia induced by BTV-1 serotype infection. The product is presented as suspension for injection.

The vaccine was eligible for the granting of a Community marketing authorisation via the centralised system in accordance with Article 3(2) of Regulation (EC) No 726/2204 (immunological veterinary medical products for the treatment of animal diseases subject to Community prophylactic measures).

The application was made in line with the requirements of the CVMP guideline on Minimum Data Requirements for an authorisation under exceptional circumstances for vaccine for emergency use against Bluetongue disease (EMA/CVMP/IWP/220193/2008).

The benefits of ZULVAC 1 Ovis are its development of an active immunisation of sheep from 1.5 months of age. The vaccine has been shown to prevent viraemia established in animals infected by BTV serotype 1. Prevention of viraemia directly benefits the animal in that this ensures reduction of clinical signs or loss of condition.

The most common side effects are a transient increase in rectal temperature, not exceeding 1.2 °C, may occur during the 24 hours following vaccination and the fact that vaccination may be followed in most animals by a local reaction at the injection site.

Epidemiology of BTV in the EU has considerably changed in the last ten years as a consequence of the incursion of different BTV serotypes in regions of the Community, where outbreaks have never been reported before and which were not considered at risk. Climate changes affecting the hydrology and ecosystems in many regions of the world, including Europe, and the finding that new species of Culicoides (such as indigenous European insects) are likely to be involved in the transmission, are suspected to have driven the spread of different BTV serotypes in Europe.

The serotype 1 (BTV-1) was first reported in Greece, in a brief epidemic in 2001. It has been demonstrated by molecular studies that the virus strain was of Eastern origin. The current epidemic in the West Mediterranean Region started after five years. According to OIE, BTV-1 was first reported in Algeria (June 2006) and a few months later in Morocco, Tunisia and Italy (Sardinia) and later it spread

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to Spain, Portugal and France. The virus strains were found close to the prototype South African BTV-1 strain (Western).

2. Quality assessment

Composition

The composition for one dose of 2 ml is provided in the following table:

Ingredients	Quantity per dose (2 ml)	Function	Reference to Standards
Active substance: Inactivated Bluetongue virus (BTV), serotype 1, strain ALG2006/01 E1	RP ≥ 1(1)	Antigen	In-house
Constituents of the adjuvant: Aluminium hydroxide hydrated for adsorption	4 mg Al^{3+}	Adjuvant	Ph. Eur. 1664
Saponin	0.4 mg	Adjuvant	In-house
Constituents of the excipient: Thiomersal	0.2 mg	Preservative	Ph. Eur. 1625
Saline solution	*q.s.* 2 ml	Diluent	In-house

(1) Relative potency by a mice potency test compared to a reference vaccine that was shown efficacious in lambs. The blending of vaccine is based on the infection titres of BTV-1 ($TCID_{50}$/ml) before inactivation.

Container

The vaccine is filled in multi-dose type I hydrolytic glass bottles (10 dose presentation (20 ml) or type II hydrolytic glass bottles (50 dose presentation (100 ml) and 120 dose presentation (240 ml)). The bottles conform with European Pharmacopoeia (Ph. Eur.) monograph 3.2.1. glass bottle specifications. They are closed with chlorobutyl rubber stopper (Ph. Eur. monograph 3.2.9) and sealed with an aluminium cap. The product is packaged together with a product information leaflet.

Development Pharmaceutics

ZULVAC 1 Ovis is an inactivated and adjuvanted vaccine for the prevention of the viraemia against Bluetongue virus serotype 1 (BTV-1). The strain originates from an Algerian outbreak in 2006 and is the same vaccine strain used for the applicant's ZULVAC 1+8 vaccine which has already obtained an authorisation under exceptional circumstances.

Sequence analyses of genome segment 2 of the Algerian BTV-1 virus shows it to be very closely related to the subsequent 'Western' isolates of BTV-1 from Europe (2007 onwards) but it is distinct from the eastern strains of BTV-1 previously isolated in Greece (in 2001). However, because of the prevalence of other BTV strains in north Africa and Europe (e.g. BTV4, and BTV-8), it is possible that the later isolates of BTV-1 from Spain, Portugal and France may have exchanged some genome segments by reassortment. Only a full genome analysis of each isolate could reveal that such an event has occurred (this has not been done). Since the identity of the European isolates that were identified as BTV-1 (after 2006) is based on Seg-2, which also determines the specificity of neutralisation and virus serotype, it can be predicted that the vaccine strain of BTV-1 from Algeria should cross-protect fully against all of the later European BTV-1 isolates, even if they are reassortants.

The virus strain was propagated in BHK-21 cells for the production of the master seed virus (MSV). The working seed virus (WSV) is prepared from the MSV. Vaccine antigens are obtained after some passages from MSV on BHK-21 cells. The virus produced is collected, titrated and inactivated with

binary ethylenimine (BEI) and, finally, the residual BEI is neutralised with sodium thiosulphate. An inactivation control test is then carried out in order to rule out presence of residual infectious virus particles.

The adjuvant system used in the formulation of the vaccine was selected according to the results obtained within the frame of the development of ZULVAC 4, an inactivated vaccine against BTV-4 which was granted an authorisation by the Spanish authorities in August 2007. Thiomersal has been used as a preservative in multi-dose containers. The saline solution is used as diluent of the antigen, and is added in sufficient quantity (q.s.) to maintain a constant quantity of antigen per dose.

A series of preliminary studies were carried out in sheep using experimental batches of a monovalent proprietary vaccine containing a different BTV serotype, in order to determine the optimal qualitative and quantitative composition in terms of adjuvants and concentration of vaccine antigen. Based on the results obtained from the series of studies, a concentration dose of vaccine antigen and a determined quantity of aluminium hydroxide and saponin were selected as target concentrations of vaccine antigen (before inactivation) and adjuvants in final batches of ZULVAC 4 vaccine. The information generated from these experiments was also taken into account for the development of other proprietary BTV vaccines, based on the fact that the same process is used for the manufacturing of the vaccine antigen, and the inactivation of BTV-1 is obtained under the same conditions established for BTV-4 and BTV-8 serotypes. In order to establish the concentration of active ingredients to be used in the formulation of the bulk vaccine, a specific dose-response efficacy study was designed in order to test the immunogenicity of the current vaccine at different concentrations of inactivated BTV-1 antigen. A pivotal efficacy study was then conducted, thus allowing to establish the minimum protective dose to prevent viraemia in all vaccinated animals with an onset immunity of 3 weeks after the completion of the primary vaccination course, in 1.5 months old lambs. A relative potency (RP) of 1 was assigned to ensure consistency in all ZULVAC 1 Ovis batches i.e. all released batches must have a RP≥1 in the validated batch potency test. The minimum and the maximum antigen content dose (at blending) has been set for BTV-1 taking into account the results of relevant safety studies.

The nature of the container materials and of the closure system has been chosen according to Ph. Eur. recommendations for injectable preparations. The vaccine will be available in 20, 50 and 120 dose presentations.

Composition of the batches used in clinical trials

Details of the vaccine preparations used in laboratory trials were provided, including details of vaccine antigen production (e.g. origin of working seed virus and working cell seed, size of antigen batches) and of manufacturing of the finished products.

Method of manufacture

The description of the manufacturing process of vaccine antigens was very detailed and precise. It took into account the majority of the comments raised for the two monovalent ZULVAC 8 Ovis/Bovis and the bivalent BTV 1+8 vaccines.

The manufacturing process consists of two steps: the production of the vaccine antigen, and the preparation of the finished product. The process starts with the propagation of the WSV and ends with the preparation of the vaccine in bulk, followed by filling and packing of the final product. The production system and control guarantees the traceability of each component during the manufacturing process. After harvest, the culture is inactivated and the inactivant neutralised at the end of the inactivation process. The bulk vaccine is further produced after blending a pre-determined amount of inactivated and neutralised vaccine antigen, thiomersal, saline solution and adjuvants.

Manufacture of vaccine antigen

Vaccine virus is grown in BHK-21 cells. The expansion of the initial cell seed is carried out in order to obtain the amount of cells needed for production. The MSV was constituted on BHK-21 cells, and stored frozen prior to vaccine production. The WSV is expanded from the MSV and also stored frozen.

In the antigen production process, the virus vaccine will be produced from the WSV by passages into BHK-21 cells.

Manufacture of the inactivated and neutralised vaccine antigen

The final viral suspension is inactivated with BEI according to Ph. Eur. requirements. The excess of inactivating agent is neutralised at the end of the inactivation process. Samples are taken from the inactivated and neutralised antigen to carry out appropriate in process controls.

Inactivated and neutralised antigen stocks can be stored at between 2° and 8 °C for a maximum of 12 months. The inactivated and neutralised antigen (active ingredient/vaccine antigen) batch size range is from 50 to 1000 litres. The batch sizes may vary based on demand for vaccine as a result of the field epidemiological situation.

Antigen from different batches may be pooled after inactivation to produce final product batches.

In order to evaluate the reliability and robustness of the manufacturing process and equivalence of antigen production in roller bottles and single use bioreactors (SUBs), a summary of critical process parameters and in-process control tests from three consecutive batches of the antigen produced in roller bottles and 3 produced in SUBs were provided. These data confirm the consistency of the production process for the inactivated BTV-1 antigen.

Manufacture of the finished product

The manufacturing process of the finished product has been described in detail and overall, is very precise. The bulk vaccine is prepared by blending pre-determined amounts of one or a mixture of several batches of inactivated and neutralised BTV antigens with thiomersal, saline solution and adjuvants. The bulk vaccine can be stored at +5.0 ± 3.0 °C for ten days until start the filling operation. Primary packaging elements (bottles and closures) are sterilized by validated cycles. Once filled, bottles of vaccine are submitted to secondary packaging operations which are carried out using a fully automatic process. The finished product is stored at +5.0 ± 3.0 °C. The vaccine bulk is normally blended and filled within 24 hours. The applicant stated that it may be decided to blend the bulk but to wait to fill the product until the results of certain tests are completed. In this case, the bulk vaccine would be stored at 2 - 8 °C until filling for no more than 10 days. This time limit is in-line with the approved process for ZULVAC 8 Ovis and ZULVAC 8 Bovis.

Validation studies

A number of studies were presented as part of the validation of the manufacturing process. The majority of the control test methods were already satisfactorily assessed during the authorisation process of the two monovalent ZULVAC 8 Ovis/Bovis vaccines. A major contribution to the validation of the manufacturing process and quality control is provided by the inactivation kinetics and by the *in vivo* batch potency test. Both types of studies were carried out for the current vaccine, as for the two aforementioned monovalent vaccines.

Inactivation kinetics

Standard inactivation kinetics studies were conducted in compliance with the Ph. Eur. requirements concerning the time required for the inactivation (not more than 67% of the duration of the inactivation process). Two studies were performed. Inactivation kinetics was tested using a BTV-1 suspension with a titre representative of routine production batches (i.e. reference batch), follow by inactivation kinetics of 10x concentrated BTV suspension. Based on the results of the studies the maximum pre-inactivation titre was established for BTV-1 serotype. Based on the results obtained, a maximum pre-inactivation titre was accepted.

The validation of the *in vivo* batch potency test was performed based on the results obtained from four doses titration studies and it has proven to be sufficiently sensitive for ZULVAC 1 Ovis as it was for ZULVAC 8 Ovis/Bovis and the bivalent ZULVAC 1+8 Ovis vaccines..

Control of starting materials

Active substance and Excipients

The documentation supplied all necessary information regarding their function, species origin and treatment before use. All starting materials except seed material of the BTV-1 antigen were already satisfactorily addressed as the current dossier was improved taking into account initial remarks raised during the authorisation process of the two monovalent ZULVAC 8 Ovis/Bovis.and the bivalent ZULVAC1+8 vaccines

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

The documentation provided demonstrate that seed materials and other starting material of animal origin relevant for the transmission of TSE, comply with the Note for Guidance on minimising the risk of transmitting animal encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 Rev2) and the corresponding Ph. Eur. monograph.

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01 Rev.2) and Commission Directive 1999/104/EEC.

Control tests during production

A detailed description was provided of the in process test controls during production. The in-process tests performed during production antigen and bulk vaccine are: virus titration, sterility, identity, inactivation, sodium thiosulphate, absence of pestiviruses, and absence of Aujeszky's Disease virus. The general characteristics, including validation where relevant, of these methods were all satisfactory assessed or appropriately adapted in the current dossier as a result of the authorisation process of two the monovalent ZULVAC 8 Ovis/Bovis vaccines. The results of the control testing carried out on three consecutive batches of BTV-1 antigen, produced using both roller flasks and bioreactors manufacturing processes, have been provided and were found compliant with the established specifications.

Control tests on the finished product

A detailed description was provided of the in process test controls on the finished product. The general characteristics of these methods, including validation where relevant, were all satisfactorily assessed

(or appropriately adapted in the current dossier) during the authorisation process of two the monovalent ZULVAC 8 Ovis/Bovis vaccines.

Data were provided in order to demonstrate that robust and consistent batches of the vaccine under application will be produced. The applicant committed to submit batch results for the first three manufacturing scale batches of the 10 dose and 120 dose presentations post-approval.

The applicant agreed to provide the results of the batch potency test (BPT) in transgenic mice carried out on (at least) the first ten batches of ZULVAC 1 Ovis vaccine. The applicant also agreed to establish an alternative *in vitro* BPT within an appropriate timeframe.

Attempts were carried out by the applicant in order to validate the current saponin test for the finished product, however up to now this has been unsuccessful. Proof of evidence of these attempts was provided. The applicant is recommended to develop an alternative test for saponin quantification however, it was not possible to give a timeline for the resolution of this specific point for concern.

The results of antimicrobial preservative effectiveness (APE) testing carried out on selected batches of vaccine was found satisfactory in order to demonstrate that the thiomersal present in the vaccine at the specific concentration, maintains its preservative activity under the conditions indicated by Ph. Eur. after 15 and at 27 months of storage.

Stability

The applicant committed to place the first three manufacturing scale batches into the long-term stability programme after approval. The results obtained to date indicate that when stored in the conditions specified in the SPC, the finished product keeps its characteristics in terms of appearance, pH, thiomersal and aluminum content for at least 9 months in the batches tested. Potency results for theses batches are only available at T0. Data are awaited in order to support the claimed stability characteristics of the vaccine under application.

Overall conclusion on quality

Overall, the quality part of the dossier can be considered sufficiently clear and complete. Quantitative and qualitative particulars of the constituents are indicated. The manufacturing method is sufficiently described. With regard to the starting materials of biological origin, details on the seed material have been completed. Seed materials and other starting material of animal origin relevant for the transmission of TSE comply with the Note for Guidance (EMEA/410/01-Rev2) and the corresponding Ph. Eur. monograph. With regard to the final product testing, the applicant has agreed to a list of recommendations that will be provided post authorisation. All this assurance was considered sufficient for granting a marketing authorisation under exceptional circumstances, but not for a full marketing authorisation.

3. Safety assessment

In order to comply with the current legislation, the safety of the administration of one dose, of repeated administration of one dose and of an overdose of the vaccine under application was assessed under laboratory conditions, in lambs of the minimum age recommended for vaccination. The safe use of the vaccine in pregnant ewes was examined in a study farm under restricted conditions. In this category of the target animal species, the safety of the administration of an overdose of the vaccine was also investigated. As allowed by the provisions in the Reflection Paper (EMEA/CVMP/IWP/105008/2007) on Minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against Bluetongue and the CVMP Guideline

(EMEA/CVMP/IWP/220193/2008) on Requirements for an authorisation under exceptional circumstances for vaccines for emergency use against Bluetongue, the aforementioned tests were carried out using a representative experimental batch of a bivalent vaccine containing BTV-1 and BTV-8. Both vaccines contain a similar amount of BTV-1 antigen, adjuvant and excipients therefore data are directly relevant.

Laboratory tests

Safety of one administration of an overdose

The administration of an overdose (4 ml) of a batch of ZULVAC 1+8 Ovis formulated at the highest vaccine antigen(s) concentration per dose was proven to be safe in lambs of minimum age. Healthy lambs which had never been vaccinated nor inoculated with other substances were enrolled for this study. The animals were allocated randomly into two treatment groups: vaccinated (V) and controls (C). In the vaccinated group (V), 13 lambs were inoculated on D0 by subcutaneous route (in the right axillary area) with a double dose (4 ml) of the vaccine under study. In the control group, seven lambs were inoculated at the same time with 4 ml of PBS. No systemic reaction was recorded, nor was general health of vaccinated animals were impaired. After the administration of an overdose of the vaccine, 85% of the vaccinated lambs (11 out 13 animals) presented local reactions at the site of injection. The reactions appeared on the 1st day after the administration of vaccine, although it was not until day 9 or 10 post vaccination when reactions were present in all reacting animals. The average duration of local reactions was of 36 days with a variation of between 12 and 48 days.

Local reactions were monitored until day 48 post vaccination, when they had disappeared in all but 5 of the reacting animals. In general the observed reactions were small granules (diameter ≤0.5cm) or oedema at the site of injection, which evolved into nodules of 1 to 4 cm in diameter (in 45% of the lambs), and in most cases into a generalised swelling of the zone of injection (in 55% of the lambs) of 2 to 9 days duration, during the most severe phase of the reaction; turning again into small granules during the resolution phase, until its total disappearance in the 55% of the lambs at the end of the study. At day 48 post vaccination, 27% of the lambs still presented with small granules of diameter ≤0.5 cm and 18% of the lambs had a nodule of 1 to 2 cm at the injection site.

The safety profile of the administration of an overdose of ZULVAC 1+8 Ovis vaccine (and as a consequence, of ZULVAC 1 Ovis) is considered to have been adequately reflected under section 4.10 of the SPC.

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

Healthy lambs which had never been vaccinated nor inoculated with other substances were enrolled for this study. The animals were allocated randomly into two treatment groups: vaccinated (V) and controls (C). In the vaccinated group (V), they were vaccinated three times 3 weeks apart (D0, D21, D42), with 2 ml of ZULVAC 1+8 Ovis, by subcutaneous route (s.c.). In the control group (C), the lambs were inoculated s.c. at the same time with 2 ml of phosphate-buffered saline (PBS).

After the administration of the three repeated doses of 2 ml of the vaccine, no systemic reactions were observed. No increases in rectal temperature were observed after the 1st vaccination, whereas after the 2nd vaccination lambs presented a transient mean rectal temperature increase of 1.19 ºC when compared to the mean temperature of the control group on day 1 after the inoculation. On day 2 after vaccination, rectal temperatures were back to normal values. A maximum temperature of 41.84 ºC was observed, with only 4 out of 13 lambs showing a transient increase in temperature above 41 ºC. After the 3rd vaccination lambs presented a transient mean rectal temperature increase of 0.65 ºC

(compared to the mean temperature of the control group) on day 1 after the inoculation. On day 2 after vaccination, rectal temperatures were normalised. A maximum temperature of 41.22 ºC was observed in a very small number of lambs with only a few showing a transient increase above 41 ºC 1 day after vaccination. A transient increase in rectal temperature after repeated vaccination is therefore considered an occasional event.

Local reactions at the injection site appeared in all vaccinated lambs after the first and second vaccinations and in 92% after the third vaccination. The average duration of the local reactions was 30 to 44 days, persisting afterwards as small granules of diameter ≤ 0.5 cm in the 54 - 61% of the lambs.

Local reactions varied from generalised diffuse swellings of the whole injection area lasting 1 to 9 days that evolved into nodules of gradually decreasing diameter from ≥2 to 0.5 cm of diameter (these local reactions appeared in 77% of the vaccinated lambs after 1st and 2nd vaccinations, and in 33% of the lambs after 3rd vaccination); to nodular swellings of ≥2 cm in diameter that gradually decreased to nodules of smaller diameter, 2 to 0.5 cm (these local reactions appeared in the 23% of the lambs after 1st and 2nd vaccinations, and in 67% of the lambs after 3rd vaccination).

At the post-mortem examination tissue reactions at the injection sites were observed: in all lambs after the first dose with a maximum volume of 0.81 cm^3 and average volume of 0.34 cm^3 and in 85 % and 92 % of the lambs after the second and third doses respectively, with a maximum volume of 2.7cm3 and average volume of 0.6 cm^3. The histopathological study showed that tissue lesions correspond to subcutaneous granuloma. The safety profile of the administration of one dose and of repeated administration of one dose of ZULVAC 1+8 Ovis vaccine (and as a consequence of the vaccine under application) is considered to have been adequately reflected under section 4.6 of the SPC.

Examination of reproductive performance

In two studies the safety of the administration in pregnant ewes at different stage of gestation of an overdose (4 ml) of ZULVAC 1+8 Ovis (formulated at the highest vaccine antigens concentration per dose) was investigated. In the first study pregnant ewes at different stage of gestation were enrolled. At the start of the study, the ewes were allocated into 3 different groups, according to the stage of gestation (groups 1, 2 and 3). Within each group, the ewes were randomly distributed into two treatment groups: vaccinated (V) and control (C). All the vaccinated ewes (V1, V2 and V3) were inoculated (D0) by subcutaneous route (s.c.) with a double dose (4 ml) of the vaccine ZULVAC 1+8 Ovis, batch E-20 and all the control ewes (C1, C2 and C3) were inoculated by s.c. route with 4 ml of PBS in the same site.

In the second study ewes at second stage of gestation (i.e. at approximately 3-5 months of gestation) were enrolled. At the start of the study, the ewes were distributed into 4 treatment groups. Groups (V1) and (V2) included ewes that were vaccinated (V1) and ewes in groups (C1) and (C2) were used as controls (C1). All ewes in V1 and V2 were inoculated (D0) by subcutaneous route (s.c.) with a double dose (4 ml) of the vaccine ZULVAC 1+8 Ovis, batch E-20, while the control ewes (C1, C2) were inoculated by s.c. route with 4 ml of PBS. Groups V1 and C1 were monitored for post vaccination reactions. All groups were monitored for reproductive performance.

Results from both studies showed that no systemic reactions were induced and the reproductive parameters of vaccinated ewes were not affected. Indeed, comparable results were obtained for vaccinated and control animals. In one of the two studies carried out in ewes at the second phase of gestation, special attention was also paid to the potential increase of rectal temperature and to the occurrence of local reactions at injection site. After vaccination, the ewes presented a transient mean rectal temperature increase of 0.6º C (with respect to the mean temperature of the control group) on day 1 after the inoculation. These data are consistent with that observed in lambs of minimum age.

After the administration of 4 ml of the vaccine ZULVAC 1+8 Ovis 83% of the ewes presented local reactions at the site of injection. The observed local reactions varied between nodular swellings of 1 to ≥ 2cm in diameter in 20% of the ewes to generalised diffuse swellings of 3 to 10 days of duration of the whole area of injection in 80% of the ewes. In 40% of the ewes the reactions persisted after day 63 post vaccination (p.v.) as small nodules of diameter ≤0.5 cm.

The safety of the vaccine was not investigated in breeding males. This circumstance is reflected under section 4.7 of the SPC.

According to the results obtained from the safety studies conducted in pregnant animals, the safety profile of ZULVAC 1+8 Ovis (and as a consequence of ZULVAC 1 Ovis) is considered to have been adequately reflected under the sections 4.6, 4.7 and 4.10 of the SPC.

The results of post-mortem inspection at the injection site carried out in studies of safety of the repeated administration of one dose of ZULVAC 1+8 OVIS to lambs, were used to reinforce the absence of any risks posed by the potential presence of residues of adjuvant system, and of thiomersal and at injection site. Therefore, no withdrawal period is required for the vaccine under application.

No data are available on the use of ZULVAC 1 Ovis with any other product; hence an appropriate warning has been included under section 4.8 of the SPC.

According to the relevant legislation, field studies can be omitted for this type of vaccine. Indeed, no field trials were performed.

User safety

The applicant has provided a detailed risk assessment concerning the potential risks put to any human beings in direct contact with the product, and derived from its components, namely the active ingredients, the adjuvant system, residues of antibiotics and preservatives. It was concluded that the nature and concentration level of the constituents are not susceptible to cause any hazard to the user. Risk of human exposure is limited to the person injecting the product to the animals (veterinarians, or experienced persons working under the direct supervision of a veterinarian), however the amount and method of administration does not pose any additional risks compared to other injectable products to animals and humans. In the absence of any demonstrated risks, according to the provisions in the User Safety Guideline for Immunological Veterinary Medicine Products (IVMPs), (EMEA/CVMP/54533/06), the recommended conditions of use are adequately reflected in the relevant section of the SPC.

Withdrawal period

The withdrawal period for ZULVAC 1 Ovis for meat and milk is zero days.

Environmental safety

An assessment of the potential harmful effects to the environment was conducted in accordance with the Note for Guidance: Environmental Risk Assessment (ERA) for Immunological Veterinary Medicinal Products (EMEA/CVMP/074/95). Due to the nature (inactivated vaccine), and composition of the vaccine under study, ERA was limited to phase I. Support to this approach relies on the following evidence. Due to the robust manufacturing process, including a fully validated inactivation test and control of inactivation, the risk arising from the presence of live Bluetongue virus in the vaccine and its excretion into the environment is considered negligible. The excipients consist of substances that will likewise not be excreted into the environment. Although ZULVAC 1 Ovis vaccine is contained in glass bottles, vial breakage or spilling of vaccine, will not lead to major consequences, as the amounts will be very small, hence the general risk posed to the environment by the use of the vaccine under study

is negligible. The first phase of the ERA assessment did not show that the use of ZULVAC 1 Ovis according to the SPC, presents a risk of exposure to the environment, hence a second phase of evaluation was regarded not necessary.

Overall conclusions on safety

Laboratory safety tests were carried out using the vaccine batch of ZULVAC 1+8 Ovis vaccine. Although the size of this batch was not standard, it can be considered representative of commercial batches as it was blended according to the standard methods described in the submitted registration dossier. The safe use of the vaccine was demonstrated according to the recommendations provided in the SPC, i.e., administration of one dose according to the following vaccination scheme: 1st injection: from 1.5 months of age, 2nd injection: after 3 weeks.

The safety profile of ZULVAC 1 Ovis vaccine was demonstrated according to current legislation. Laboratory studies with the vaccine were provided. Under the tested conditions, the vaccine was generally well tolerated as demonstrated by the absence of major systemic reactions impacting body temperature and growth performance following administration in sheep. Local reactions were acceptable in terms of size, frequency of occurrence, and duration. Studies regarding the reproductive performance were provided that were conducted on pregnant ewes; no impact on the offspring was reported. The safe use of the vaccine in breeding males was not investigated and references to that have been noted in the relevant section of the SPC. Additional data were provided regarding the selection of the animals enrolled in the safety studies which ensured the reliability of the results obtained from the concerned studies. Evidence was provided that showed that the risk for the environment and for the user is negligible. Standard safety advice has been inserted in the SPC

Overall, the safety profile of ZULVAC 1 Ovis vaccine was demonstrated. The potential for any adverse effects following the administration of the vaccine under the recommended conditions of use is adequately reflected in the relevant section of the SPC.

4. Efficacy assessment

Introduction and general requirements

ZULVAC 1 Ovis is recommended for the active immunisation of sheep in order to prevent viraemia established in animals infected by BTV serotype 1. A 2 ml dose of the vaccine is recommended to be administered by subcutaneous route to sheep (including pregnant animals). The basic vaccination schedule consists of one initial injection given from a minimum of 1.5 months of age and followed by a second injection given 3 weeks later. Field trials were not strictly required for this type of application. A differentiation of infected from vaccinated animals (DIVA) strategy has not yet been implemented.

A summary of the vaccine batches used in laboratory efficacy studies was provided and they were considered appropriate for the use in efficacy studies.

Since it is expected that BTV vaccines are capable to prevent viraemia, the following definition was agreed to substantiate the claim for protection against BTV viraemia: consistent absence of viral load detectable by real time qRT-PCR (segment 5, Toussaint et al, 2007) in all the vaccinated animals during the monitoring period of minimum 4 weeks. Viral load detectable by real time qRT-PCR was defined as the one that provides a result of Ct value lower than 36.0 in vaccinated animals (thus predicting the interruption of virus transmission). During the qRT-PCR validation studies, its sensitivity in virus suspension was 2 TCID50/ml for BTV-1.

To establish a suitable challenge model an amount of virus was inoculated so that all control animals become viraemic during the study (from approximately 5 days to 27 days post infection). Challenge

models were established for BTV-1. The strain is considered relevant based on the sequence information available. Data available through Pharmacovigilance would support the efficacy of the selected vaccine strain. The absence of (or minimal) clinical signs in lambs challenged with BTV-1 (and BTV-8) has also been observed in previous ZULVAC studies (ZULVAC 8 Ovis, ZULVAC 1+8 Ovis). Evidence was provided that it is a problem of the experimental model. However, absence of viraemia is an indication of no clinical disease and no infectivity for insects. The indication "prevention of viraemia" is acceptable. Although several procedures are currently used to detect BTV in blood samples or tissues of infected animals, these procedures are laborious and time consuming. As an alternative, a validated qRT-PCR was used for detection of BTV genome in experimentally challenged animals. The results obtained were then correlated to infectious virus titres in cell culture. It has been demonstrated that the test is repeatable and reproducible for serotype 1. The limit of detection for BTV-1 is 10-1.4 (equivalent to 101.0 TCID50/ml of blood).

Laboratory studies

The results from one pre-immunogenicity study conducted with experimental vaccine batch preparations of ZULVAC 1 Ovis provided supportive evidence of the expected efficacy of the vaccine under application. A brief summary of this study is provided below.

Study of the efficacy and safety of the vaccine ZULVAC 1 Ovis, formulated at different concentrations of BTV in 1-month-old lambs

The objective of this GLP study was to evaluate the efficacy of three different antigen concentrations in ZULVAC 1 Ovis vaccine in order to establish the lowest vaccine concentration able to prevent viraemia (presence of genome in blood) in vaccinated lambs.

The three different batches of ZULVAC 1 Ovis were formulated at different antigen concentrations per dose, Vaccine 1, 2 and 3 at 2 ml per dose.

One month old lambs, without antibodies against BTV were included in the study and randomly allocated into four treatment groups:

Group 1: lambs, vaccinated and revaccinated with ZULVAC 1 Ovis, Vaccine 1.
Group 2: lambs, vaccinated and revaccinated with ZULVAC 1 Ovis, Vaccine 2.
Group 3: lambs, vaccinated and revaccinated with ZULVAC 1 Ovis, Vaccine 3.
Group 4: control lambs, not vaccinated.

Groups 1, 2 and 3 lambs were vaccinated with 2 ml by subcutaneous route (s.c.) and revaccinated 3 weeks later.

Lambs of group 4 were left as unvaccinated controls. After each vaccination, the lambs were monitored for the appearance of any systemic reactions associated with the administration of the vaccine (anaphylactic shock, anorexia, vomiting, etc.).

Twenty-four days after revaccination, animals of each vaccinated group and animals in control group were challenged subcutaneously in the left axilla, with 2 ml of bluetongue virus serotype 1 (BTV-1). They were monitored daily during 15 days post-infection, for appearance of clinical signs associated with the disease (rectal temperature, nasal and/or ocular discharge, nasal and/or ocular oedema, other oedemas, lameness, prostration and death).

The primary parameter taken into account in order to support the efficacy of each vaccine preparation was the capacity to prevent presence of the viral genome in blood in the vaccinated and challenged lambs, as verified by means of the validated, aforementioned qRT-PCR.

Before 1st vaccination (D.0), 2 weeks after revaccination (D+35), and 1 day before challenge (D+44), blood samples were taken from all the lambs (vaccinated and controls) in order to evaluate the animals' serological status and response after the administration of vaccine.

Blood samples were taken from the animals on days 3, 5, 7, 8, 11, 14, 18, 21, 25 and 28 post challenge (p.c.), for evaluation of the presence of the BTV genome.

Results showed that none of the lambs manifested any systemic reactions (anaphylactic shock) after 1st and 2nd vaccination. There were no statistically significant differences regarding rectal temperature between the three vaccinated groups and the control group after the 1st and 2nd vaccination.

Local reactions in the three vaccinated groups were very similar. The most severe results were regarding the number of animals with local reactions, and the duration and intensity of the reactions were described.

Before the 1st vaccination neither ELISA nor SN antibodies were detected in any animals of any groups. Whereas controls remained seronegative at every time point, 2 weeks after revaccination (D35) and the day before challenge (D44), ELISA and SN antibodies were detected in all the vaccinated groups.

In none of the vaccinated lambs (groups 1, 2 and 3) challenged with BTV-1, was viral genome detected during 28 days after challenge, whereas in all the non-vaccinated (group 4) and challenged lambs the viral genome was detected from D3 post infection (p.i.).

Control lambs manifested a statistically significant rectal temperature increase in contrast with the vaccinated groups on days 3, 5, 6, 7, 8, 9, 10 and 11 p.i.

Control lambs presented a statistically significant higher clinical signs score from day 5 until the end of the observation period after challenge (D 15 p.i.). Also the total clinical signs score after challenge was significantly higher in the control lambs compared to the vaccinated groups.

The results of this study are considered just of supportive interest for the efficacy of the vaccine. Additional, relevant information and comments were provided concerning the selection of the animals enrolled in the study (randomisation criteria), the preparation and use of the experimental vaccine (pre-immuno vaccine batch BTV-1-A), and the challenge virus strain.

Minimum protective dose/onset of immunity

The results from this study, conducted with monovalent ZULVAC 1 Ovis, undiluted and 1:2 diluted provided evidence that the blending of the selected vaccine antigen concentrations and of the mixture of the two adjuvants was efficacious starting 21 days after the completion of the recommended vaccination scheme. A brief summary of these studies is provided below.

Study of efficacy of ZULVAC 1 Ovis vaccine in 1.5-month-old lambs

The objective of this GLP study was to evaluate the efficacy of ZULVAC 1 Ovis, to prevent viraemia (presence of viral genome in the blood) in 100% of the vaccinated and challenged lambs. The final goal was to establish the minimum protective dose and the onset of immunity.

In this study, the batch reference (representative of commercial batches of ZULVAC 1 Ovis vaccine), was tested undiluted and diluted 1:2 with a placebo buffer composed of aluminum hydroxide and saponin.

Six weeks old lambs, without antibodies against BTV were included in the study. They were randomly allocated into three treatment groups:

Group 1: with lambs, vaccinated and revaccinated with ZULVAC 1 Ovis, Batch reference
Group 2: with lambs, vaccinated and revaccinated with ZULVAC 1 Ovis, Batch reference diluted 1:2
Group 3: with control lambs, not vaccinated

According to the recommended vaccination scheme and administration, lambs of groups 1 and 2 were vaccinated (D0) with 2 ml by subcutaneous route (s.c.) and revaccinated 3 weeks later (D21). Lambs of group 3 were left as unvaccinated controls.

Twenty-one days after revaccination (D42), the groups were challenged with virulent BTV-1 with 2 ml of a viral suspension. The primary parameter selected to demonstrate the efficacy of the two vaccine preparations was the capacity to prevent the presence of the viral genome in blood in the vaccinated and challenged lambs, as verified by means of a validated qRT-PCR.

After each vaccination, the lambs were monitored for the appearance of any systemic reaction associated with the administration of vaccine (anaphylactic shock, anorexia, etc.).

Before 1st vaccination (D0) and at challenge (D42), blood samples were taken from all the lambs in order to evaluate the serological status (by SN). After challenge, blood samples were taken from the animals on D0, 2, 4, 7, 10, 14, 17, 21, 24 and 28 post infection, for the evaluation of the presence of the BTV genome.

The animals were monitored on D0, 2, 4, 7, 10, 14, 17, 21, 24 and 28 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, prostration and death).

The results of ZULVAC 1 Ovis batch reference, tested undiluted and diluted 1:2, induced in vaccinated lambs an active immunity able to prevent viraemia after the challenge. Viral genome was not detected in any of the vaccinated lambs, during 28 days after challenge with BTV-1, whereas in all unvaccinated/challenged lambs, the viral genome was detected from D3 after challenge.

Statistical significant differences were recorded between the vaccinated groups and the control group on day's 4-10 post infection.

Statistical significant differences (Mann-Whitney U-test) were not recorded between the vaccinated and the control lambs, however nasal and ocular and/or ocular oedema were the only clinical signs recorded just before the death of 2 control lambs out of 6 (25%) died as a consequence of the experimental challenge.

In addition, this study provides further safety data to show that the administration of ZULVAC 1 Ovis vaccine to 1.5-month-old lambs did not provoke any general reactions (anaphylactic shock).

The efficacy of the vaccine in the face of Maternally Derived Antibodies (MDA) has not been investigated. A warning has been included in the relevant section of SPC.

Duration of immunity

The results from one study conducted with monovalent ZULVAC 1 Ovis provided evidence that the vaccine should be able to induce the claimed protection for up to 1 year after completion of the primary vaccination scheme. A brief summary of this study is provided below.

Study of the duration of immunity of ZULVAC 1 Ovis vaccine in 1.5 month-old lambs

The objective of this study was to verify the efficacy of ZULVAC 1 Ovis vaccine to prevent viraemia in sheep challenged 1 year after the completion of the basic 2 shots vaccination scheme.

The study initially included a group of 1.5-month old healthy lambs, without antibodies against BTV. They were challenged 1 year (i.e. D375) after completion of the basic vaccination scheme. None of the lambs manifested any systemic reactions (anaphylactic shocks and/or vomiting) after 1st and 2nd vaccinations.

Before vaccination (D<0), none of the lambs selected for the study presented ELISA antibodies against any of the BTV serotypes.

The results of the geometric mean neutralising antibody titres (GMT) against BTV-1 in vaccinated lambs were reported. Control lambs were negative (GMT<2) at all bleeding times. Values ranged from 53.4 to 241.6 at day 42.

In none of the vaccinated sheep challenged with BTV serotype 1, viral genome was detected by the validated qRT-PCR during 4 weeks after challenge, whereas in all the controls, viral genome was detected from D5 after challenge.

Statistically significant differences were found regarding the rectal temperatures between the control and the vaccinated group on days 5, 7 and 10 after challenge. These time points corresponded with the days of maximal viraemia when the controls/challenged animals presented a higher rectal temperature than the vaccinated/challenged animals.

After the BTV-1 infection very mild and not specific clinical signs were recorded. Clarification was provided regarding the challenge inoculum and the clinical outcome of the experimental infection.

Field trials

Data on field trials were not provided. According to the CVMP guideline on minimum data requirements for an authorisation under exceptional circumstances for vaccines for emergency use against Bluetongue disease, specific field trials are not a required

Overall conclusion on efficacy

The efficacy data provided are considered sufficient to support the proposed indication for the active immunisation to protect lambs from 1.5 months of age against BTV serotype 1 infection and to prevent viraemia. The onset of immunity is 21 days after completion of the primary vaccination course, and duration of immunity is 12 months. Laboratory efficacy trials supported this claim.

In the absence of any specific data, and consistent with the approach followed for other ZULVAC BTV serotype monovalent and bivalent vaccines, the following statement was agreed to be used: any revaccination scheme should be agreed by the Competent Authority or by the responsible veterinarian, taking into account the local epidemiological situation.

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

5. Benefit risk assessment

Introduction

ZULVAC 1 Ovis is a conventionally produced, liquid and ready-to-use, binary ethylenimine (BEI) inactivated, and aluminium hydroxide/saponin adjuvanted vaccines, against bluetongue virus (BTV) serotype 1 infection.

Direct therapeutic benefit

The benefit of the product is active immunisation of sheep from 1.5 months of age against infection with BTV serotype 1. The vaccine has been proven to prevent viraemia. Onset of immunity is 3 weeks after the completion of the basic vaccination course. The duration of immunity is 12 months.

Indirect or additional benefits

In addition to the direct benefit to the vaccinated animal (active immunisation and prevention of viraemia), there is a benefit to herd health both locally and regionally.

The use of vaccines such as ZULVAC 1 Ovis is important at a Community animal health level as they are the most effective way to control disease spread as there are no efficient ways to control the insect vector and no therapeutic treatment for BTV infections. Vaccination has been shown to be an efficient tool for disease control.

The extent to which prevention of viraemia is achieved would be likely to block virus transmission. Therefore, the vaccination of sheep with ZULVAC 1 Ovis can be considered as a valuable tool in the control programme against BTV and for the "safe" trade of live target animals according to OIE rules or EU legislation.

Risk assessment

ZULVAC 1 Ovis is well tolerated by the target animals. No significant risks were identified when the product is used as indicated in the SPC and under normal veterinary practice conditions. The potential risks posed by the use of the vaccine in terms of strictly safety issues and of lack of efficacy, have been satisfactorily addressed by the inclusion of clear warnings in relevant sections of SPC, thereby mitigating any remain risk to an acceptable level.

According to the risk assessment provided, the active ingredient and excipients do not present a risk to the user and to the environment.

An environmental risk assessment is provided demonstrating that ZULVAC 1 Ovis contains no ingredients which are considered harmful to the environment.

Any risk to the consumer with respect to vaccines given to food producing species relate to any residual live organism or vaccines residues in meat. As ZULVAC 1 Ovis is inactivated there are no risks of residual live virus. With respect to residues from vaccination, it has been demonstrated that there are no residues left in meat which would present a risk to the consumer.

Conclusion on benefit risk balance

The information provided in the dossier (quality safety and efficacy) and in response to points raised is sufficient to confirm an overall positive benefit risk balance under exceptional circumstances. No significant risks were identified when the product is used as indicated in SPC and under normal veterinary practice conditions. This conclusion is also supported by the experience gained from the positive outcome of the authorisation process of the two monovalent vaccines ZULVAC 8 Ovis/Bovis.

ZULVAC 1 Ovis has shown to be efficacious for the active immunisation of sheep from 1.5 months of age for the prevention of viraemia caused by BTV-1. Onset and duration of immunity is at 3 weeks and 12 months, respectively, after the completion of the basic vaccination.

Conclusion

The CVMP considered that due to the current epidemiological situation of bluetongue regarding serotype 1 and the consequent threat to animal health there are objective and verifiable reasons for recommending the granting of a marketing authorisation under exceptional circumstances for this product, namely:

- that Bluetongue disease is spread by insect vectors and therefore presents particular challenges in terms of control due to an inability to prevent transmission from infected animals other than through insect control combined with reducing or preventing viraemia (virus in the blood) in susceptible animals by means of vaccination;
- that Bluetongue disease is epizootic in nature and has the potential to result in high morbidity and mortality in sheep;
- that there is a remaining epidemiological risk from Bluetongue serotype 1 (BTV1) for European sheep populations, in view of recent and previous outbreaks of BTV1 in Europe that constitute an objective need to have authorised products available for use in the coming months;
- that consequently any delay should be avoided where possible in making available safe and effective vaccines that have been demonstrated to be in compliance with the CVMP guideline on Minimum Data Requirements for an authorisation under exceptional circumstances for vaccines for emergency use against Bluetongue (EMEA/CVMP/IWP/220193/2008);
- that the application has met the requirements of the CVMP guideline on Minimum Data Requirements for an authorisation under exceptional circumstances for vaccines for emergency use against Bluetongue (EMEA/CVMP/IWP/220193/2008);
- that the applicant has agreed to the necessary specific obligations, to assure the safe use of the product in the field.

The applicant cannot reasonably be expected to provide the results from certain trials on the target species due to the difficulties in conducting large scale trials for a disease that is under community control and the need for any experimental studies to be conducted within high containment facilities.

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