Institute for State Control of Veterinary Biologicals and Medicines
Ústav pro státníkontroluveterinárníchbiopreparátů a léčiv

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MUTUAL RECOGNITIONPROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Borrelym 3, suspension for injection for dogs
(in CZ, EE, HU, LT, LV, PL, RO, SI, SK)
Merilym 3, suspension for injection for dogs
(in AT, BE, DE, FR, IE, IT, LU, NL, PT, UK)
Trilyme, suspension for injection for dogs
(in DK, FI, NO, SE)

MODULE 1

PRODUCT SUMMARY

EU Procedure number	CZ/V/0114/001/MR
Name, strength and pharmaceutical form	Borrelym 3, suspension for injection for dogs (in CZ, EE, HU, LT, LV, PL, RO, SI, SK) Merilym 3, suspension for injection for dogs (in AT, BE, DE, FR, IE, IT, LU, NL, PT, UK) Trilyme, suspension for injection for dogs (in DK, FI, NO, SE)
Applicant	Borrelym 3: Bioveta, a.s., Komenského 212, 683 23 IvanovicenaHané, Czech Republic Merilym 3/Trilyme: Merial 29 Avenue Tony Garnier, 69007 Lyon, France
Active substance(s)	Inactivated Borrelia burgdorferi sensu lato: Borrelia garinii Borrelia afzelii Borrelia burgdorferi sensu stricto
ATCvetcode	QI07AB04
Target species	Dogs
Indication for use	For active immunization of dogs from 12 weeks of age, to induce an anti-OspA response against *Borrelia spp.* (*B. burgdorferisensustricto*, *B. garinii*and *B. afzelii*). Reduction of *Borrelia* transmission was only investigated under laboratory conditions, following a challenge with field ticks (collected from a region known to be affected by *Borrelia*). Under these conditions, it was shown that no *Borrelia* could be isolated from the skin of vaccinated dogs, while *Borrelia*were isolated from the skin of non vaccinated dogs. Reduction of transmission of *Borrelia* from the tick to the host has not been quantified, and no correlation has been established between a specific level of antibodies and reduction of *Borrelia* transmission. The efficacy of the vaccine against an infection that leads to the development of clinical disease has not been studied.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Veterinary Medicines Agencies website (http://www.HMA.eu).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Mutual Recognition application in accordance with Article 31 of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	20/12/2012
Date product first authorised in the ReferenceMemberState (MRP only)	16/04/2009
Concerned Member States for original procedure	AT, BE, DE, DK, EE, FI, FR, HU, IE, IT, LT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK

I. SCIENTIFIC OVERVIEW

The product is produced and controlled using validated methods and tests, which ensure the consistency of the product released on the market.

It has been shown that the product can be safely used in the target species; the reactions observed are indicated in the SPC.

The product is safe for the user and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.

The efficacy of the product was demonstrated according to the claims made in the SPC.

The overall risk/benefit analysis is in favour of granting a marketing authorisation.

II. QUALITY ASPECTS

A. Composition

Composition:

Active substances:

Inactivated *Borrelia burgdorferi sensu lato*:
Borrelia garinii ..... RP ≥ 1*
Borrelia afzelii..... RP ≥ 1*
Borrelia burgdorferi sensu stricto ..... RP ≥ 1*

*RP = Relative potency (ELISA test) compared with the reference serum obtained after vaccination of mice with a vaccine batch that has successfully passed the challenge test in the target species.

List of excipients:

Aluminium hydroxide hydrated for adsorption
Formaldehyde
Sodium chloride
Potassium dihydrogen phosphate
Disodium hydrogen phosphate dodecahydrate
Water for injection

The vaccine is presented in hydrolytic class I glass vials. The vials are sealed with pierceable rubber stoppers and secured with aluminium caps. Glass vials are packed in plastic boxes.

A) Plastic box with 10 wells:
10 x 1 ml of the vaccine
2 x 1 ml of the vaccine
B) Plastic box with 20 wells:
20 x 1 ml of the vaccine
C) Plastic box with 100 wells:
100 x 1 ml of the vaccine
50 x 1 ml of the vaccine

The particulars of the containers and controls performed are provided and conform to the regulation of monographs 3.2.1 and 3.2.9 of the European Pharmacopoeia.

The choice of thevaccine strains, of the vaccine composition, adjuvant, inactivating agent, absence of preservative, of the dose volume and vaccination schedule are justified.

The inactivation process and the detection limit of the control of inactivation are correctly validated.

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site.A corresponding manufacturing licence and GMP certificates are provided.

Process validation data on the product have been presented in accordance with the relevant European guidelines.

The product is manufactured in accordance with the European Pharmacopoeia and relevant European guidelines.

C. Control of Starting Materials

Starting materials of non-biological origin used in production comply with indicated pharmacopoeia monographs.

Biological starting materials used are in compliance with the relevant Ph. Eur. Monographs and guidelines and are appropriately screened and appropriately treated for the absence of extraneous agents according to the Ph. Eur monographs.

The master and working seeds have been produced according to the Seed Lot System as described in the relevant guideline and satisfactorily tested according to current Europea requirements.

Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

Scientific data and/or certificates of suitability issued by the EDQM have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

D. Control tests during production

The tests performed during production (growth and purity control, density control, presence of OspA antigens, inactivation control, serum absence control, sterility test, pH determination, aluminium content determination) are described in detail and the results of 3 consecutive runs, conforming to the specifications, are provided.

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E. Control Tests on the Finished Product

The tests performed on the final product conform to the relevant requirements; any deviation from these requirements is justified. Relevant validations are provided.

The tests include in particular :

- appearance
- content of the original package
- sterility
- identity
- potency
- pHvalue
- content of aluminium oxide
- formaldehyde
- air tightness

The demonstration of the batch to batch consistency is based on the results of 3 batchesproduced according to the method described in the dossier.

F. Batch to batch consistency

The consistency of production has been demonstrated and the results of 3 consecutive runs, conforming to the specifications, are provided.

G. Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substances (inactivated antigens 3 months, after concentration and purification further 6 months and bulk of the vaccine 1 month before filling) when stored under the approved conditions (2-8°C).

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life (2years) when stored under the approved conditions (2-8°C).

III. SAFETY ASSESSMENT

Safety clinical findings have been based on the recommended vaccination scheme of two doses of 1 ml Borrelym 3 /Merilym 3 /Trilyme vaccine dose, 3 weeks apart, administered by the subcutaneous route to dogs and using maximum potency. The recommended minimum age for vaccination is 12 weeks.

Safety studies have been performed with a vaccine batch (containing maximum content of all antigens) produced according the described production process.

Laboratory trials

The safety of the administration of one dose, an overdose and the repeated administration of one dose in the target animal is demonstrated in controlled laboratory studies which in total included 30vaccinates animals (12-week-old puppies). The investigation was performed according to the recommendations of Directive 2001/82/EC as amended and the relevant guidelines.

The safety studies demonstrate that the administration of one dose, an overdose, and the repeated administration of a dose can be considered to be safe, when used in accordance with the recommended vaccination schedule. Some minor, transient adverse reactions were observed following vaccination.Appropriate warning regarding local site reactions following vaccination have been included in the SPC: Vaccination may induce a transient increase in body temperature (not more than 1.5°C). Transitory swelling may be observed through palpation at the injection site (maximum diameter of 7 mm for a maximum of 5 days).

Vaccine is not intended for administration to pregnant and / or lactating animals. Therefore, no specific studies were performed. The proposed text of SPC reflects this claim.

There are no data suggesting that this product might adversely affect the immune system of the vaccinated animal or its progeny therefore a specific study was not carried out.

With regards to the adjuvant, aluminium hydroxide is added at the final formulation of the product as an adjuvant to a final concentration of 2.0 mg/ml. Aluminium hydroxide is widely used in veterinary and human vaccines as adjuvant at this or similar concentrations.

Consequently, it has been concluded that the inclusion of this material does not justify specific studies of residues.

No specific assessment of the interaction of this product with other medicinal product was made. Therefore, an appropriate warning in the SPC is included.

Field studies

Combined safety and efficacy field trial was performed on target animals.

30 animals were selected, 20 of them were vaccinated and 10 were kept as controls. A batch of the vaccine, containing average titres of the antigens, produced by the method described in the marketing authorisation documentation was used in the study. Animals were vaccinated according to the vaccination schedule recommended in Summary of Product Characteristics. Safety of the vaccine was evaluated based on:

a) Monitoring of systemic reactions after vaccination and re-vaccination
b) Monitoring of local reactions in vaccination spot
c) Monitoring of body temperature
Animals were observed one day prior to vaccination and 14 days after vaccination and re-vaccination.

Injection site reactions in the form of swelling in some cases sensitive to palpation but not inflammatory with a maximum diameter of 3 mm were observed in dogs after vaccine administration. Local reactions were observed in six dogs after vaccination and re-vaccination. There was no increase in rectal temperatures in any of the animals after vaccination.

Therefore, the administration of Borrelym 3 /Merilym 3 /Trilyme vaccine by the recommended route under field conditions and according to vaccination schedule was found to be safe for target species. The results obtained reflected those observed in the laboratory safety studies.

This safety profile was also confirmed by Pharmacovigilance data from current use of this product.

Ecotoxicity

The applicant provided a first phase environmental risk assessment in compliance with the relevant guideline which showed that no further assessment is required.

The conclusions of the environmental risk assessment as presented by the applicant, that there is a very low risk to the environment associated with use of the vaccine, are accepted. The applicant has included the standard disposal statement for inactivated vaccines on the product literature and this is considered acceptable.

IV. EFFICACY

All trials performed to demonstrate efficacy of Borrelym 3 /Merilym 3 /Trilyme vaccine were designed to comply with the requirements of relevant European veterinary legislation including the European Directive 2001/82/EC as amended and relevant European Pharmacopoeia chapters in force.

The efficacy on the target species dogs was demonstrated in animalsat the minimum age recommended for vaccination (12 weeks).The batches of minimum declared potency used in the trials were manufactured using the procedure described in the marketing authorisation

documentation. Onset and duration of immunity was proved in target animals on the basis of infection agent re-isolation and serology.

Animals were challenged by natural way of infection. Naturally infected ticks were used as a vector of the infection agent. The percentage of infection was determined using PCR.

Laboratory Trials

The efficacy of the product has been demonstrated in laboratory studies in accordance with the relevant requirements.

Onset of immunity

Forty animals at the age of twelve weeks, seronegative for OspA antigen, were included in the study on onset of immunity. Twenty animals were administered recommended dose (1 ml) of Borrelym 3 /Merilym 3 /Trilyme using a batch containing minimum antigen titre on days 0 and 21 of the study. Twenty animals were vaccinated with placebo containing adjuvant in phosphate saline buffer.
The animals were challenged on day 49 of the study. Naturally infected ticks were used as a vector of the infection agent. A representative sample of 118 ticks from the tick population was examined to confirm the presence of *Borrelia*. The percentage of infection was determined using species-specific PCR. Nineteen ticks were found infected with at least one strain of *Borrelia*. The evaluated *Borrelia* infection rate was 16,1%. The infestation groups of vectors were prepared and placed in a chamber. One chamber containing 50 ticks was placed on each side of animal thorax. The chambers were controlled on the days 23 and 26 and the attached ticks were counted. The chambers were definitely removed on the day 29 and the ticks were left in the place.
The dogs were observed daily after challenge for clinical signs of the disease. Body temperature was measured daily from day 41 until the end of the post-challenge monitoring period (day 133).
The body weight of all dogs was monitored on days 0 (prior to vaccination), 21 (prior to second dose administration) and regularly during the challenge phase on days 49, 63, 77, 91, 105, 119 and 133.
Blood samples for serological examination were withdrawn on days 0, 21, 49, 63, 77, 91, 105, 119 and 133. The samples were assayed for OspA titres in a single ELISA assay for each time point.
Biopsies were collected aseptically from all dogs from the area covered by the tick enclosure device after the challenge on days 57, 77, 105, 133. The necropsy and tissue sampling was carried out in every animal at the termination of the trial.
Biopsy samples and tissues samples from necropsy were subjected to infection agent re-isolation and PCR examination (by two independent laboratories). Serum samples from the blood collection on days D0, D49, D77 and D133 were subjected to Western Blot analysis.

Clinical symptoms of the disease were observed neither in the vaccinated group nor in the control group. Rectal temperatures of the animals were within the physiological limits during the period of observation.
The vaccine Borrelym 3 /Merilym 3 /Trilyme induced production of protective OspA antibodies after the performed immunization. Anti OspA antibodies against all serovars contained in the vaccine were detected in dogs of the vaccinated group. The dogs of non-vaccinated groups were without detectable values of anti OspA antibodies.
All samples (skin biopsy samples and tissues after necropsy) of vaccinated dogs examined by cultivation method were negative for Borrelia. The positive isolations of Borrelia were achieved from some skin biopsy samples in the non-vaccinated group: 11 positive on day 57, 8 positive on day 77, 3 positive on day 105. The isolations from skin biopsy samples obtained on day 133 and all isolations from necropsy samples were negative.

PCR detection showed that all samples (skin biopsy samples and tissues after necropsy) of the vaccinated dogs were negative for Borrelia DNA. Positive PCR detection of Borrelia DNA was registered in some skin biopsy samples in the non-vaccinated group: 20 positive on day 57, 14 positive on day 77, 5 positive on day 105 and 1 positive on day 133. PCR detection of Borrelia in all necropsy samples was negative. Samples from the study were shipped to an independent expert laboratory for confirmative testing, using species-specific PCR (more sensitive). All vaccinates were confirmed negative for Borrelia sl. The results obtained confirm all Borrelia strain presence in the controls. Only one biopsy sample of control animals was positive for Borreliab.ss.
The formation of anti-OspA response against *Borrelia spp*. and reduction of *Borrelia* transmission was proved four weeks after basic vaccination by challenge.

Duration of immunity

Ten animals at the age of three months, free of antibodies against all antigens contained in the vaccine were included in the laboratory efficacy study. Animals were of the minimum age recommended for vaccination.
Eight animals were administered basic vaccination (2 doses, interval between vaccinations was 14 days) with 1 ml of vaccine batch containing minimum antigen titer, two animals were used as controls. Animals were challenged one year after revaccination. Natural way of infection by ticks was used. Prior to infection, a representative sample from the tick population was examined to confirm the presence of B*orrelia*. Infection rate was 20%.
Animals were monitored for 60 days after infection. Animals were observed for clinical symptoms (apathy, dysorexia, limping, crisp hair, touch painful) and re-isolation of challenge organisms from bioptic samples was performed. Blood samples were collected to measure level of specific anti-OspA antibodies, which are known to be protective against Borrelia infection.

No significant clinical symptoms in the vaccinated or control dogs were observed during two months of monitoring after the challenge. This fact can be caused by a relatively short period of the monitoring after the challenge with respect to the character of borreliosis development (chronic symptoms) in host and with respect to clinical symptoms manifestation.
The presence of Borrelia in control non-vaccinated dogs was demonstrated by bacteriological examination in lymph node on the left side (dogs no. K1, K2) and in muscle on the right side (dog no. K2). Borrelia were re-isolated from most of the skin samples of control dogs. Borrelias were not detected in the samples collected from the vaccinated dogs.
PCR detected the presence of spirochaetes in skin samples and all lymph nodes of non-vaccinated animals, right muscle of one control animal (K1) and both muscles of the other control (K2).
The protection of the vaccinated dogs against infectious agents during the challenge experiment was examined also by the serological tests (detection of antibodies using ELISA method). Increased titres of OspA antibodies against Borrelia infection were evidenced. High levels of anti-OspA antibodies were observed in the vaccinated dogs during all-time monitoring. Anti-OspA antibodies were not detected in non-vaccinated dogs. The levels of anti-OspA antibodies correlate with the results from re-isolation and PCR detection. Both re-isolation and serological examinations proved the duration of immunity in dogs12 months after the challenge.

The following claimed indications for Borrelym 3 /Merilym 3 /Trilyme are considered to be supported by the laboratory studies:

For active immunization of dogs from 12 weeks of age, to induce an anti-OspA response against Borrelia spp. (B. burgdorferi sensu stricto, B. garinii and B. afzelii).

Reduction of Borrelia transmission was only investigated under laboratory conditions, following a challenge with field ticks (collected from a region known to be affected by

Borrelia). Under these conditions, it was shown that no Borrelia could be isolated from the skin of vaccinated dogs, while Borrelia were isolated from the skin of non vaccinated dogs.

Reduction of transmission of Borrelia from the tick to the host has not been quantified, and no correlation has been established between a specific level of antibodies and reduction of Borrelia transmission. The efficacy of the vaccine against an infection that leads to the development of clinical disease has not been studied.

Onset of immunity: 1 month after primary vaccination.

Duration of immunity: one year after primary vaccination.

Field studies

Combined safety and efficacy field trial was performed on target animals.

30 animals were selected, 20 of them were vaccinated and 10 were kept as controls. A batch of vaccine containing average titres of the antigens, produced by the method described in the marketing authorisation documentation was used in the study. Animals were vaccinated according to the vaccination schedule recommended in Summary of Product Characteristics.

The immunology profile has been monitored by a system of four blood sampling in all indicated animals – initial status before vaccination, after three weeks = before revaccination, then sampling between the first and second month following revaccination, and the last sampling after one year before revaccination.

Investigation of antibodies against Borrelia antigens contained in the vaccine was carried out using optimised ELISA method.

A positive seroconversion was observed after administration of the first and the second dose of the vaccine in comparison with the non-vaccinated control group. A significant increase of anti-OspA antibodies in vaccinated animals was observed 1 – 3 months after Borrelym 3 /Merilym 3 /Trilyme administration. High levels of anti-OspA antibodies persisted in vaccinated animals at least for 12 months from revaccination. No changes in antibody levels were observed in control animals. The results obtained in these studies confirm the findings obtained in the laboratory trials.

V. OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the risk benefit profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.