EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines and Product Data Management

Scientific discussion

This module reflects the initial scientific discussion for the approval of RHINISENG (as published in July 2010). For information on changes after this date please refer to module 8.

1. Summary of the dossier

Inactivated vaccine against atrophic rhinitis in pigs.

On 14 July 2010 the Committee for Medicinal Products for Veterinary Use (CVMP) adopted a positive opinion,[**] recommending the granting of a marketing authorisation for the veterinary medicinal product RHINISENG, suspension for injection.

The applicant for this veterinary medicinal product is Laboratorios Hipra S.A..

The active substance of RHINISENG is
Inactivated *Bordetella bronchiseptica*, strain 833CER: 9.8 BbCC(*)
Recombinant Type D *Pasteurella multocida* toxin (PMTr): ≥ 1 MED63(**)
(*) *Bordetella bronchiseptica* Cell Count in log10.
(**) Murine Effective Dose 63: vaccination of mice with 0.2 ml of a 5-fold diluted vaccine by subcutaneous route induces seroconversion in at least 63% of the animals.

The main benefits of RHINISENG are the passive protection of piglets via colostrum after active immunisation of sows and gilts to reduce the clinical signs and lesions of progressive and non-progressive atrophic rhinitis, as well as to reduce weight loss associated with *Bordetella bronchiseptica* and *Pasteurella multocida* infections during the fattening period. Challenge studies have demonstrated that passive immunity lasts until piglets are 6 weeks of age while in clinical field trials, the beneficial effects of vaccination (reduction in nasal lesion score and weight loss) are observed until slaughter.

The most common side effects are transient local reactions which may occur after the administration of one dose of vaccine. A transient slight swelling of less than 2 to 3 cm in diameter is common at the injection site which may last up to five days and occasionally up to two weeks.

A transient increase in body temperature of about 0.7°C is common during the first 6 hours after injection. An increase of rectal temperature up to 1.5ºC may occur after a singe dose administration. This rectal temperature increase is spontaneously resolved within 24 hours without treatment.

[**] Applicants may appeal any CVMP opinion, provided they notify the EMA in writing of their intention to appeal within 15 days of receipt of the opinion.

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2. Quality assessment

Composition

RHINISENG is presented as a suspension for injection in vials containing 2 ml per dose. The active substances are inactivated *Bordetella bronchiseptica* strain 833CER and the recombinant Type D *Pasteurella multocida* toxin (PMTr). A combination of DEAE-Dextran, ginseng and aluminium hydroxide is used as adjuvant, and simethicone, formaldehyde and phosphate buffered saline (PBS) solution as excipient. Further details on the qualitative and quantitative composition are listed in the SPC.

DEAE-Dextran and aluminium hydroxide have been used in the manufacture of different inactivated vaccines for swine and other species. However, its combination with ginseng constitutes an innovative aspect in the veterinary medicine.

Ginseng is a plant of the family of Araliaceae widely used in East Asia. The main active components of ginseng roots are triterpenoic saponins and ginsenosides. At least 25 ginsenosides have been identified and are present in variable amounts and ratios to one another, depending on the particular species, variety and conditions of growth. Extensive investigations on the toxicity of ginseng extract confirmed by clinical trials demonstrate that ginseng is absolutely safe under the conditions of use and that the pharmacological effects or efficacy are plausible on the basis of long-standing use and experience. In addition, the adjuvant effect of ginseng has been widely demonstrated.

The three adjuvant components are either included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 or considered not within the scope of Regulation (EC) No 470/2009 at the dose included in RHINISENG. The choice of formaldehyde as antimicrobial preservative is acceptable and the efficacy of the preservative has been shown to be satisfactory.

Container

The vaccine is filled into colourless glass Type I (20 ml) and Type II (50 ml and 100 ml) and polyethylene terephthalate (PET) injection bottles (20, 50, 100 and 250 ml) closed with bromobutyl rubber stoppers. The glass containers are heat-sterilised in the oven. The PET containers are gamma-irradiated and the stoppers are steam sterilised.

Development Pharmaceutics

The choice of the antigens, adjuvant, preservative, containers and stoppers etc. is well described.

Method of manufacture

The manufacturing process for the inactivated *Bordetella bronchiseptica*, strain 833CER consists of the following steps:

- Culture in plates
- Preparation of the pre-inoculum
- Preparation of the inoculum
- Culture in fermenter
- Inactivation
- Concentration

The manufacturing process of the recombinant Type D *Pasteurella multocida* toxin consists of the following steps:

- Culture in plates

- Preparation of the pre-inoculum
- Preparation of the inoculum
- Culture in fermenter
- Concentration
- Cell lysis
- Purification of the PMTr by chromatography
- Sterilising filtration

The manufacturing process of the final vaccine RHINISENG consists of the following steps:

- Preparation and sterilisation of the aqueous phase
- Preparation of the final suspension
- Filling
- Labelling

The description of the manufacturing process and methods is clear and detailed. The manufacturing processes of the antigens and final vaccine are appropriately validated.

The inactivation time used for inactivation of the *B. bronchiseptica* antigen is demonstrated suitable.

Control of starting materials

Active substance

The inactivated and resuspended *B. bronchiseptica* antigen is tested for bacterial and fungal sterility, concentration of total bacteria, residual formaldehyde and pH.

The concentrated PMTr antigen is tested for bacterial and fungal sterility, identity PMTr, concentration PMTr and pH.

Stability of the antigens

Two batches of *B. bronchiseptica* and three batches of PMTr were stored for the proposed storage period at 2-8°C and used to manufacture three different vaccine batches filled in 20 ml glass bottles and 20 ml PET bottles. Each antigen batch was submitted to the in-process control tests detailed below at the beginning and at the end of the proposed storage period before being used for vaccine manufacture. The results for the antigen testing met the specifications established for the bulk antigens.

The proposed storage time of the two antigens at 2-8°C is acceptable.

Excipients

Certificates of analysis were provided for all adjuvants/excipients and their components. These materials were tested according to Ph.Eur. or internal procedures.

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

Starting material of biological origin	Origin of material by species
Bordetella bronchiseptica 833CER inactivated	Porcine
Recombinant Type D *Pasteurella multocida* toxin (PMTr)	Produced in *E. coli*
Blood agar plates (Columbia sheep blood agar)	Bovine/ovine
Yeast extract	Yeast
Tryptone	Bovine milk
Lysozyme	Avian
Glutathione	Yeast
Gelatine	Porcine

The starting materials of biological origin comply with the 'NfG on minimising the risk of transmitting animal spongiform encephalopathies agents via human and veterinary medicinal products' (EMEA/410/01-Rev02). The risk of transmission of Animal Spongiform Encephalopathies is considered negligible.

Control tests during production

The in-process tests performed during production of the *B. bronchiseptica* 833CER antigen are: gram stain, viability/purity, turbidity, pH, identity, count of viable colonies, concentration of total bacteria, inactivation, bacterial and fungal sterility and residual formaldehyde.

The in-process tests performed during production of the recombinant Type D *P. multocida* toxin (PMTr) antigen are: gram stain, viability/purity, PCR, pH, turbidity, count of viable colonies, count of total bacteria, concentration of PMT, identity PMTr, purity, bacterial and fungal sterility.

The in-process tests are adequately described and satisfactorily validated according to the VICH guidelines on validation of analytical procedures (CVMP/VICH/591/98 and CVMP/VICH/590/98).

Consistency of production has been demonstrated by testing several production scale batches of antigens and final vaccine.

Control tests on the finished product

The methods used for the control of the finished product (appearance, pH, concentration of formaldehyde, concentration of aluminium hydroxide, concentration of ginsenosides, concentration of DEAE-Dextran, identity of the antigens, inactivation, sterility, bacterial endotoxin, safety, potency (*B. bronchiseptica* antigen concentration), potency, packaging and volume control) are well described and satisfactorily validated according to the VICH guidelines.

The specifications proposed at release and end-of-shelf life are appropriate to control the quality of the product.

The PMTr potency test is performed as an alternative potency test in mice using a serological test. The test is considered a relevant indicator of the efficacy of the final vaccine as correlation to the immunogenicity in target species has been demonstrated. The test is satisfactorily described and validated and the ability to detect subpotent batches has been demonstrated.

The *B. bronchiseptica* potency test is performed as a combination of an *in vitro* method for total bacteria counting and a serological test determining the response against the Bb. antigen in mice. This combination of tests is sufficiently justified and considered suitable for determination of the potency of the *B. bronchiseptica* antigen.

The results of the analysis of three pilot scale batches and two production scale batches were presented; all specifications were met.

Stability

Stability of the finished product

An interim report is provided on the ongoing real time stability study presenting stability data for nine vaccine batches filled in colourless glass bottles (20, 50 and 100 ml) and six vaccine batches filled in PET bottles (20 and 250 ml)) stored at 2-8°C for up to 27 months. The following parameters were assessed at appropriate intervals: appearance, pH, bacterial and fungal sterility, volume, concentration of aluminium hydroxide, concentration of ginsenosides, concentration of formaldehyde, endotoxins, potency and safety. The 50 ml glass bottles were only tested at T0, T12, T21 and T27. The results for up to 27 months comply with the end-of-shelf-life specifications except for the results of the activity test PMT (*in vivo*) and the serological *B. bronchiseptica* test for which the results are pending. The proposed shelf life of 24 months at 2°C-8°C is considered acceptable with a commitment made to submit the 27 months results for the activity test PMT (*in vivo*) and the serological *B. bronchiseptica* test as soon as they are available.

In-use stability

Samples from six vaccine batches, three of them filled in 100 ml colourless glass containers and three in 250 ml PET containers. All samples were pierced and stored at room temperature (20°C) for 10 hours and then tested at suitable intervals over the proposed in use shelf life for sterility and potency. The choice of the biggest presentations was justified by the fact that under field conditions, bigger presentations are more likely to remain broached and susceptible to become contaminated or lose activity. The results remain within the specifications.

The in-use stability was also assessed in the test on efficacy of formaldehyde as antimicrobial preservative in which one vaccine batch was inoculated with different inocula of bacteria and fungi and stored at 20-25°C for 10 hours. Samples were drawn at suitable intervals over the proposed in-use shelf life to determine the number of cfu/ml. One vaccine batch without formaldehyde was added as negative control. Results obtained for vaccine batches stored for up to 24 months demonstrate that the preservative efficacy is maintained during the in-use shelf life. The proposed in-use shelf life of 10 hours at 15-25°C is acceptable.

Environmental risk assessment for products containing or consisting of genetically modified organisms

As this vaccine does not contain a GMO capable of replicating in the environment but a properly defined not viable recombinant protein, this part is not applicable for the evaluation of the product in question.

Overall conclusions on quality

To conclude, several concerns were identified during the initial assessment of RHINISENG. These are all resolved.

3. Safety

Safety documentation

Laboratory tests

Safety of the administration of one dose

Enclosed in the dossier two trials evaluated the safety of administration of one dose. All studies were conducted in compliance with GLP.

The first laboratory study assessed the safety, efficacy and duration of immunity, with respect to the basic vaccination and revaccination schemes for RHINISENG. Two single doses were administered to pregnant sows at 8 and 4 weeks before farrowing. After 1st farrowing the same sows were mated again and a single booster dose was administered 4 weeks before the expected farrowing date for 2nd farrowing. An equal number of sows served as controls and they were administered PBS at the same vaccination schedule as for the RHINISENG vaccinates. Rectal temperatures were recorded from all sows the day before vaccination, at the day of vaccination and 2, 4 and 6 hours later and daily for four days. Local and systemic reactions were monitored intensively for a 14 day period after each administration thereafter daily until the end of the study. Reproductive parameters from each gestation period were also recorded. Results showed a significant increase in temperature at 6 hours post vaccination, but the mean temperature returned to normal within 24 hours and the average values did not exceed 1.5°C. Slight local reactions were recorded at the injection site but they were not followed by inflammation. No remarkable general adverse reactions were observed. No abortions were recorded and the main reproductive parameters were unaffected by vaccinations.

The second laboratory study was identical to the study above, except for the fact that the administration of the basic vaccine took place at 6 weeks and 3 weeks before expected farrowing and the efficacy of the booster dose was not assessed. The results also were similar to those observed in the previous study. In conclusion the safety after single administration of RHINISENG was documented.

Safety of one administration of an overdose and repeated administration of one dose

In order to diminish the number of animals used for both the safety of one overdose and of one repeated dose in line with Council Directive 86/609, the safety study protocols for a double dose and repeated single doses were combined.

The safety of administration of a double dose and two single doses was assessed in a laboratory trial. In this study primiparous sows aged 6 months were administered 4 ml of RHINISENG and 21 and 35 days post vaccination the sows were revaccinated with a single dose (2 ml). The animals were observed until farrowing and the parameters observed rectal temperatures measured the day before vaccination, the day of vaccination, 2, 4 and 6 hours post vaccination and daily for the next 4 days. Local, systemic reactions and reproductive performance were also recorded. Animals were euthanised 7 days after farrowing and histological analysis from the injection site was made.

The average temperature increase did not exceed 1.5°C after any administration, and no animals showed a rise higher than 2°C. The maximum rise in temperature was recorded 6 hours post vaccination, but they were not associated with systemic reactions and returned to normal values within 24 hours. Acceptable macroscopic local reactions were recorded, mainly after administration of the third dose. The highest proportion of gilts (6 out of 10) showed mild local inflammation at the injection

site, but reactions did not persist for more than a week. Histology revealed small granulomata attributed to aluminium hydroxide. Such a lesion was considered as normal for adjuvanted vaccines. Reproductive performance was considered being not affected and normal for sows.

The same laboratory study was repeated using a vaccine batch manufactured at pilot scale and containing the maximum concentration of endotoxins. This study included vaccinated sows and controls administered only PBS. The results obtained from this study were comparable to those obtained from the previous study. It could be concluded that administration of a double dose of RHINISENG was safe.

One laboratory study assessed the safety and possible adverse reproductive effects of RHINISENG in boars. One overdose and repeated administration of one dose was administered to boars by the same vaccination scheme as applied in the previous studies. Rectal temperatures were measured the day before vaccination, the day of vaccination, 2, 4 and 6 hours post vaccination and daily for the next four days. Local and systemic reactions were recorded for 14 days post vaccination. The reproductive parameter (semen quality) was assessed. Results were in line with those of sows with respect to increase in rectal temperature 6 hours after administration. Rise in temperature did not exceed 2^{o}C and returned to normal values within 24 hours. The quality of semen was evaluated in terms of percentage of primary abnormalities (e.g. normal acrosomes, % total motility; % progressive motility). Secondary abnormalities recorded were morphological abnormalities such as % abnormal heads, abnormal neck, distal droplets, proximal droplets and abnormal tails. No adverse reactions were recorded in any of the semen quality parameters.

Examination of reproductive performance

The reproductive performance was investigated in the gilts and sows included under single dose, overdose and repeated dose administration discussed above. No reproductive disturbances were reported in any of these studies.

Examination of immunological functions

Examination of the immunological functions as required by Directive 2001/82/EC was not performed, since RHINISENG is an inactivated vaccine and therefore no adverse effects on the immunological functions are expected.

Study of residues

The adjuvant components of RHINISENG are: aluminium hydroxide, DEAE-dextran and ginseng. Formaldehyde is used as inactivating agent for the *Bordetella bronchiseptica* antigen and is also added as preservative for the final suspension. Aluminium hydroxide, ginseng and formaldehyde are substances included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010.

The DEAE-dextran is a polysaccharide naturally occurring such as celluloses and hydroxycelluloses, dextrans and glucans, and is therefore included in the list of substances considered as not falling within the scope of Regulation (EU) No 470/2009. Consequently, it is considered that there is no need to perform residue studies for the vaccine RHINISENG.

Interactions

No documentation was provided, and the SPC contains the standard sentence regarding this matter.

Field studies

Safety of RHINISENG under field conditions was assessed in one multi-centre field study. The field study was conducted in three farms sited in North-East of Spain and included healthy pregnant gilts and sows of different parities. The animals were randomly divided into 2 groups, and approximately half of the sows were vaccinated according to the basic vaccination and revaccination scheme, while the other pregnant animals were vaccinated with placebo (PBS). Adverse reactions were monitored in sows from both groups for two consecutive farrowings. Rectal temperatures were measured in all included sows before each vaccination, on the day of treatment, 6 hours after vaccination and daily for 3 consecutive days. The general health status and the local reactions were observed during the first three days post vaccination then every week until farrowing.

Results demonstrated that vaccination of sows according to the vaccination programme did not provoke unacceptable local or systemic reactions after vaccination. A total of 52.5 % of RHINISENG vaccinated sows showed a transient local reaction sized less than 1 cm in diameter which disappeared after 48 hours in most cases. The percentage of local reactions decreased to 11.1 % in relation to the second administration, and lesions had disappeared 7 days later in all animals. Local swellings sized 2-3 cm in diameter were observed in 21.2 % of vaccinated sows after administration of the 1st dose. These swellings disappeared within 48 hours in most animals. Swellings occurred in 8 % of sows post administration of the second dose, and no swellings were recorded after the booster dose.

No unacceptable rises in temperature were recorded after the first vaccination (Max. increase 0.4^{o}C). Significant differences between treatment groups were observed six hours after administration of the 2nd and booster dose, but results were within acceptable values.

No significant differences in reproductive parameters between the two treatments were recorded.

In general it was concluded that RHINISENG was safe when administered under laboratory and field conditions to the target species. The SPC, Section 4.6 and 4.10, was updated according to the above results and the current SPC guideline.

User safety

RHINISENG is a suspension for parenteral administration to be administered by intramuscular injection. The vaccine is composed of inactivated whole cells of *Bordetella bronchiseptica* and a non-toxic derivative of Type D *Pasteurella multocida Toxin* (PMTr) produced by recombinant methods as antigen components, aluminium hydroxide, DEAE-Dextran and ginseng as adjuvants and simethicone, formaldehyde and phosphate buffered saline (PBS) solution as excipient.

The antigen components of RHINISENG do not pose any risk to the person handling the product. The antigen *Bordetella bronchiseptica* is properly inactivated during the manufacturing process and the inactivation control tests ensure that no viable micro organisms are present in the finished product. The antigen PMTr is a non-toxic protein that is produced using a host-vector system composed of a genetically modified strain of *Escherichia coli* that is properly eliminated during the purification process. The rest of the ingredients are reported not to be harmful for human beings.

The vaccine RHINISENG is bottled in tamper-proof airtight containers to ensure that the product would not come into contact with possible contaminants or the person handling the product during ordinary conditions of handling storage and transport.

No user safety concerns are related to this vaccine, as the composition does not contain any substances that could involve any particular risk for the person handling this product. The standard

sentence proposed in the SPC was modified to reflect the real (small) risk after accidental self-injection.

Environmental risk assessment

An environmental risk assessment (ERA) phase I was performed, which demonstrated the vaccine as having no estimated risk for the environment. Therefore a Phase II ecotoxicity study was considered unnecessary.

Overall conclusion on safety

The safety of RHINISENG was demonstrated in several well conducted and reported laboratory and field studies. Results demonstrated that the vaccination of sows, gilts and boars according to the vaccination programme did not provoke unacceptable local or systemic reactions after vaccination and no significant differences in reproductive parameters between vaccinated and placebo-treated animals were recorded either.

The SPC, Section 4.6 and 4.10 has correctly been updated with the adverse reactions recorded in accordance with the current SPC guideline and reflecting all the known information from the dossier especially with respect to incidence and duration of adverse reactions.

An ERA Phase I study was performed, which demonstrated the product as having no estimated risk for the environment. Therefore a Phase II study was considered unnecessary. The vaccine does not pose any risk to the person handling it, as it is properly inactivated and bottled in tamper-proof airtight containers. The demands on user safety are fulfilled. The SPC standard warning has been confirmed.

Overall, the safety of this vaccine has been well documented and reported for the target animals, the user, the consumer and the environment.

4. Efficacy

Introduction and General Requirements

RHINISENG is an inactivated vaccine with two active ingredients; i.e. whole-cell antigen of *Bordetella bronchiseptica* strain 833CER and the recombinant Type D *Pasteurella multocida* toxin (PMTr) produced in a host-cell system composed of a genetically modified strain of *Escherichia coli*. Non-active ingredients are aluminium hydroxide, DEAE-dextran and ginseng as components of the adjuvant system, and simethicone, formaldehyde and phosphate buffered saline solution as diluent vehicle.

The indication for RHINISENG is passive immunisation of piglets via colostrum, after the active immunisation of sows and gilts, to reduce the clinical signs and lesions of progressive and non-progressive atrophic rhinitis.

Atrophic rhinitis is an important contagious respiratory disease of pigs that causes high economic losses to the pig industry related to impaired growth performance in affected animals. The clinical signs include sneezing, twisting and shortening of the nose due to underlying atrophy of the nasal turbinate bones, and retarded growth rate. Infection with *Bordetella bronchiseptica* alone causes non-progressive atrophic rhinitis giving mild to moderate turbinate atrophy and also assists colonisation of the nasal cavity by large numbers of toxigenic *Pasteurella multocida* which are required to reproduce severe and progressive atrophic rhinitis. Clinical progressive atrophic rhinitis (PAR) is usually controlled by a combined *Bordetella bronchiseptica*/ *Pasteurella multocida* vaccination.

The major virulence factor associated with PAR is the *Pasteurella multocida* toxin (PMT), which is encoded by the toxA gene. PMT is one of the major factors contributing to the pathogenesis of PAR, as it inhibits osteoblast differentiation and bone formation.

The innovative fact introduced in the RHINISENG vaccine lays on the composition of its adjuvant fraction as a combination of aluminium hydroxide, DEAE-dextran and Ginseng. All studies were conducted in compliance with GLP.

Laboratory trials

Establishment of a challenge model

The European Pharmacopoeia monograph on inactivated vaccines against porcine atrophic rhinitis describes in detail the challenge model to be applied in efficacy trials. The conditions under which the challenge is carried out shall mimic the natural conditions for infection. According to Ph. Eur. the challenge test would be invalid if fewer than 80 percent of the progeny of each litter of unvaccinated sows have a total Nasal Lesion Score (NLS) of at least 10. The applicant established a challenge model presented in 3 laboratory studies. One pathogenic *Bordetella bronchiseptica* strain (BP 21) and two pathogenic *Pasteurella multocida* strains (PP-12 and Pm 1990) were included.

The first study indicated that the *B. bronchiseptica* BP-21 strain was able to induce atrohpic rhinitis lesions but the PM 1990 *P. multocida* strain was not adequate to constantly meet the demands stated in Ph. Eur.In the other 2 challenge studies performed, the conditions to comply with the Ph. Eur. requirements were established for both *B. bronchiseptica* BP-21 strain and PM 1990 *P. multocida* strain.

Laboratory efficacy studies

Once the challenge model was established two preliminary efficacy studies were carried out during the development of the vaccine.

The laboratory study was the first approach to determine the safety and efficacy of the RHINISENG vaccine. An experimental vaccine formulated in the same way as the final RHINISENG vaccine was used. Two groups of pregnant sows were used and the first group was vaccinated with a single dose at 8 and 4 weeks before farrowing, whereas the second group of sows acted as unvaccinated controls. Safety parameters including rectal temperatures, local and general reactions and reproductive parameters were monitored in both groups of sows. An additional group of sows was treated with a double dose at 8 weeks before farrowing and revaccinated four weeks after with a single dose. Piglets from all sows were allowed to be fed by their own mother until 5 days of age. Then two challenge groups (Bp + Pm and PM) were made including an equal number of piglets from vaccinated and unvaccinated sows. The Pm group was challenged by the intranasal route at 10 days of age with a toxigenic strain of *P. multocida*. The Bb + Pm group was challenged at 7 days of age with 1 *B. bronchiseptica* and *P. multocida*. On the 2 consecutive days preceding challenge the mucosa of the nasal cavity of all piglets was irritated by instillation of a acetic acid solution. A third challenge group (sentinels) composed of piglets (from vaccinated and unvaccinated sows) was also established.

At the age of 42 days all piglets were euthanised, and the nose was dissected transversally at premolar-1. The ventral and dorsal turbinates and the nasal septum were examined for evidence of atrophy or deviation of the nasal septum. Results showed that regardless the challenge group, the mean NLS in piglets from vaccinated sows was significantly lower than that recorded in piglets from control sows. The serological data from the sows showed good transmission of passive immunity to the offspring.

The next laboratory study was intended to assess both the safety and efficacy of different experimental formulations based on the antigen and adjuvant composition of RHINISENG. Different modifications in the PMTr concentration and inclusion of different excipients were assessed. The different concentrations of PMTr were intended to assess whether the efficacy against atrophic rhinitis was proportional to the PMTr concentration. Four groups of sows were vaccinated with 2 single doses of experimental vaccines A, B, C and D (vaccine A corresponded to the final formulation of RHINISENG)

administered 6 and 3 weeks before farrowing. The fifth group consisted of sows administered a double dose of vaccine D 6 weeks before farrowing and a single dose 3 weeks before expected farrowing. The sixth group of sows was administered PBS. All groups were monitored as described in the previous study. After farrowing the same challenge protocol as described in the previous study was established. In this study the challenge groups Bb + Pm and Pm were composed of an equal number of piglets from each treatment group Including also sentinels. Results showed that all vaccine formulations were safe and effective, and no statistically significant differences between vaccinated groups could be detected.

Duration of immunity

One laboratory study assessed both the efficacy and duration of immunity, both with respect to the basic vaccination and revaccination schemes for RHINISENG. Two single doses were administered to pregnant sows at 8 and 4 weeks before farrowing. After 1st farrowing the same sows were mated again and a single booster dose was administered 4 weeks before the expected farrowing date for 2nd farrowing. An equal number of sows were included as controls and administered PBS at the same vaccination schedule as for the RHINISENG vaccinates. From birth until 5 days of age piglets were fed by their own mothers. Then two challenge groups of piglets were made each group consisting of piglets from a representative group of sows, taking not fewer than 3 piglets from each litter. On the 2 consecutive days preceding challenge the mucosa of the nasal cavity of the piglets was irritated by instillation of acetic acid in isotonic buffered saline solution. At 7 days of age challenge was made with a toxigenic *B. bronchiseptica* to the Bb + Pm group, and the same piglets were challenged at 10 days of age with a toxigenic strain of *P. multocida* (doses according to the challenge model).

An additional group of piglets from vaccinated and unvaccinated sows remained as sentinels. All piglets were euthanised at an age of 42 days, and the nose was dissected transversally at premolar-1. The ventral and dorsal turbinates and the nasal septum were examined for evidence of atrophy or distortion of the nasal septum. Results showed that regardless of the challenge group, the mean NLS recorded in piglets from vaccinated sows was statistically significantly lower than that recorded in piglets from control sows. The challenge model was valid by the fact that more than 80% of control piglets showed a nasal lesion score higher than 10. Mortality rates did not differ between the groups. Significant differences in antibody titres measured in colostrum samples from vaccinated sows and control sows were observed. The results of piglets correlated with the serological status of their mothers.

Another laboratory study was identical to the study above, except for the fact that the administration of the basic vaccine took place at 6 weeks and 3 weeks before expected farrowing and the efficacy of the booster dose was not assessed. The results also were similar to those observed in the previous study.

Field trials

One multi-centre field study was carried out to assess both the safety and efficacy of RHINISENG under field conditions. The field study involved 3 farms with pregnant gilts and sows at different parities. The animals were randomly allocated into two groups: the first group consisted of sows vaccinated according to the recommended vaccination programme (6 weeks and 3 weeks before farrowing) and a booster dose 3 weeks before the expected date of the subsequent farrowing. The second group of pregnant sows were vaccinated with placebo (PBS) according to the same vaccination schedule.

Results included nasal lesion score as the primary response, and secondary responses were clinical signs in pigs until slaughter, serological response against *B. bronchiseptica* and PMTr in sows at different time points, bacteriological isolations from nasal and tonsil swabs, and production responses in terms of age until slaughter and daily growth.

The results supported those obtained from the laboratory studies as a clear seroconversion against both antigens was observed in the vaccinated sows. This immune response was transferred to the offspring via colostrum, and a reduction in the NLS was reported in piglets euthanised at 6 weeks of age, but the NLS also remained lower in this group of pigs from vaccinated sows until slaughter. The age at slaughter was reduced by 3 days in comparison to pigs from the PBS-vaccinated control group.

Overall conclusion on efficacy

The efficacy of RHINISENG was demonstrated for the category of the target species for which the vaccine is recommended (gilts and sows) by the recommended route of administration (intramuscular) using the proposed schedule of administration (first vaccination at 6-8 weeks before farrowing and revaccination 3 weeks later). The studies from the original dossier support the indication "progressive atrophic rhinitis" and a major point was asked in order to support the claim for reduction of "non-progressive" atrophic rhinitis. In the answers to the list of questions the applicant submitted further support for this, and the arguments were accepted. The efficacy of vaccination of boars was not documented from data in the dossier, therefore this target species was removed from the proposed SPC, Section 4.1.

The antigen concentration in RHINISENG is standard and the vaccine batches used had been produced according to the manufacturing process described in the dossier.

The efficacy results supported those obtained from the laboratory studies as a clear seroconversion against both antigens was observed in the vaccinated sows. This immune response was transferred to the offspring via colostrum, and a reduction in the NLS was reported in piglets euthanised at 6 weeks of age, but the NLS remained lower in this group of pigs from vaccinated sows until slaughter. The age at slaughter was reduced by 3 days in comparison to pigs from the PBS-vaccinated control group.

Part 5 – Benefit Risk Assessment

Introduction

RHINISENG is presented as a suspension for injection in vials containing 2 ml per dose. The active substances are inactivated *Bordetella bronchiseptica* strain 833CER and the recombinant Type D *Pasteurella multocida* toxin (PMTr). The claim proposed by the applicant is: *"For passive immunisation of piglets via colostrum from sows and gilts actively immunised with the vaccine to prevent the clinical signs and lesions of progressive and non-progressive atrophic rhinitis"* and the proposed target species are *"sows and gilts".*

Benefit assessment

The safety and efficacy of RHINISENG have been demonstrated in well conducted GLP-trials that fulfil the requirements of Directive 2001/82/EC, as amended, and the relevant monograph of the European Pharmacopoeia.

Direct therapeutic benefit

Well-conducted placebo-controlled clinical studies demonstrated that vaccination of pregnant sows or gilts with RHINISENG reduces the incidence and severity of progressive and non-progressive atrophic rhinitis in growing pigs. The vaccine has a new adjuvant, which includes ginseng as immune modulator principle. However, the efficacy of vaccination of boars in the herd was not demonstrated therefore they were excluded as target species. Likewise the claim for the vaccine to "prevent" clinical signs was changed to "reduce" clinical signs in order to reflect data in the dossier correctly.

Additional benefits

As a consequence of reduced atrophic rhinitis disease, the incidence of respiratory symptoms and subsequent treatment with antibiotics of the pigs is also reduced. The pigs, which have received antibodies against atrophic rhinitis via colostrum, need a shorter time to reach the slaughter weight.

Risk assessment

The risk using this inactivated vaccine can be classified as minimal.

Main potential risks for the vaccine as such:

- for the target animal: there are mild and transitory local reactions at the injection site, resolving within few days and a transiently elevated body temperature within acceptable limits. This is reflected in the relevant sections of the SPC.
- for the user: accidental self-injection is the only identified risk and an appropriate warning has been included in the SPC to reflect the (small) risk.
- for the environment: No risk identified from the use of this inactivated vaccine
- for the consumer: All components has been investigated and no risk for the consumer has been identified.

Risk management or mitigation measures

The SPC contains warnings in the relevant sections.

Evaluation of the benefit risk balance

The product is well tolerated by the target animals and presents a low risk for users and the environment. The efficacy has been demonstrated for the target species "sows and gilts" and the agreed wording of the claim is:

"Piglets: For the passive protection of piglets via colostrum after active immunisation of sows and gilts to reduce the clinical signs and lesions of progressive and non-progressive atrophic rhinitis, as well as to reduce weight loss associated with Bordetella bronchiseptica and Pasteurella multocida infections during the fattening period.

Challenge studies have demonstrated that passive immunity lasts until piglets are 6 weeks of age while in clinical field trials, the beneficial effects of vaccination (reduction in nasal lesion score and weight loss) are observed until slaughter."

Conclusion on benefit risk balance

It can be concluded that the benefits provided to the target animals by the vaccination using RHINISENG, outweigh the risks for the target animals, the user, the environment and the consumer.

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considers that the application for RHINISENG for the passive protection of piglets via colostrum after active immunisation of sows and gilts to reduce the clinical signs and lesions of progressive and non-progressive atrophic rhinitis, as well as to reduce weight loss associated with *Bordetella bronchiseptica* and *Pasteurella multocida* infections during the fattening period is approvable.