Scientific Discussion for
MS-H VACCINE (EMEA/V/C/000161)

Introduction

Further to the submission of a letter of intent by Pharmsure Ltd on 23 February 2009, the CVMP accepted on 15-17 April 2009 that MS-H vaccine was eligible for the submission of a dossier for granting of a Community marketing authorisation via the centralised procedure The Committee confirmed that MS-H vaccine was eligible under Article 3.2(a) of the Regulation EC No 726/2004, as the product contains a new biological active substance, which was not authorised in the Community on the date of entry into force of the Regulation. The CVMP confirmed that the product MS-H vaccine is considered minor use according to the Guideline on Data Requirements for Immunological Veterinary Medicinal Products Intended for Minor Use or Minor Species/Limited Markets (EMEA/CVMP/IWP/123243/2006-Rev.1). The company Pharmsure Ltd. has SME status.

Pharmsure Ltd submitted an application to the EMEA on 26 October 2009 for the granting of a Community marketing authorisation in accordance with Article 31 of Regulation (EC) No 726/2004 of 31 March 2004.

An application for the granting of a Community marketing authorisation of MS-H vaccine has been submitted to the European Medicines Agency on 26 October 2009 by Pharmsure Ltd in accordance with Regulation (EC) No. 726/2004. MS-H vaccine contains a live *Mycoplasma synoviae* MS-H strain and is presented in packs/containers of 1,000 doses. It is indicated for active immunisation of future broiler breeder chickens, future layer breeder chickens and future layer chickens to reduce air sac lesions and reduce the number of eggs with abnormal shell formation caused by *Mycoplasma synoviae*. The route of administration is ocular use. The target species is chicken.

Part 1 - Administrative particulars

The finished product is manufactured by Bioproperties Limited at their Glenorie Vaccine Manufacturing Facility in Australia. This site has been inspected by the Therapeutics Goods Administration (TGA), the regulatory authority in charge of GMP auditing in Australia, a country where Mutual Recognition Agreements with the European Community apply. The GMP status of all sites involved in the manufacture of this product was confirmed as satisfactory.

A pharmacovigilance system is in place and a DDPS has been provided.

Overall conclusions on administrative particulars

The administrative particulars provided were satisfactory.

Part 2 - Quality

Composition

Mycoplasma synoviae, Strain MS-H, living …….≥$10^{5.7}$ CCU/dose

Modified Frey Medium containing swine serum……30 µl

The MS-H vaccine is presented as 1,000 doses in a nominal 30 ml bottle containing live frozen vaccine in which the active component consists of viable *Mycoplasma synoviae*.

Container

The container is a 30 ml colourless translucent bottle of Low Density Polyethylene (LDPE) without additive, capped with a grey butyl rubber stopper and an aluminium cap. All these materials are gamma irradiated.

Development Pharmaceutics

The presentation of the vaccine and the route of administration (eyedrop) are adequately justified. The mutant strain has been selected in the University of Melbourne. The temperature sensitivity (ts) is most likely not the only mutation causing the attenuation. Therefore, the stability profile of the vaccinal strain was supported not only by following the ts phenotype but also by serial passages through birds. These *in vivo* data are presented in the Safety section.

Method of manufacture

The manufacturing process is particularly simple and straightforward. It consists of two main steps: 1) growth of the mycoplasma culture (inoculation of the working seeds, fermentation and 2) preparation of the finished product (harvest, filling, capping, labelling and packaging, and storage at -70°C). The process is well described, including information on the dilutions at each passage, the batch size, the relation between mycoplasma growth phase and medium pH, the incubation periods etc.

Control of starting materials

The starting materials used for manufacture of the vaccine are sufficiently controlled by their specifications and Certificates of Analysis are provided for each material. The viral safety of starting materials of biological origin is ensured by appropriate measures, based on sound risk assessments. In this regards, a risk assessment on the use of non-irradiated swine serum in the manufacture of the seeds allows concluding that the viral risk, if any, is negligible. As for the irradiation of swine serum used in the manufacturing process, it has been increased up to ≥ 30 kGy, ensuring high safety level without detrimental effect on the growth serum profile.

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

The TSE risk has been adequately addressed and is considered negligible.

Control tests during production

The control tests during production include a purity test by gram staining, a purity test by direct inoculation onto rich media agar plates, pH measurements and container fill volume check.

Control tests on the finished product

The testing of the finished product includes potency, identity (temperature sensitivity), appearance, pH, freedom from *Mycoplasma gallisepticum*, identity (PCR), safety and sterility. The proposed maximum release titre is acceptable.

Batch to batch consistency

The demonstration of batch-to-batch consistency was conducted on 3 non-consecutive batches. Such an approach is not in line with the Note for guidance on process validation but is deemed acceptable given the MUMS/limited market status of the vaccine and the recommendation given in the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets.

Stability

The proposed shelf life is for up to 4 years at or below -70°C, followed by short term storage for up to 4 weeks at or below -18°C. This short term storage is necessary since on farm, storage at or below -70°C is not achievable. It is also proposed that the product leaflet recommends that, following rapid thawing, the product be handled and administered to chickens within 2 hours. As a consequence, stability testing was undertaken in three parts, i.e. stability of the final product when stored at -70°C, stability of the final product when placed in -18°C storage and "in-use" stability of the product, after rapid thawing and storage at room temperature.

The vaccine proved to be remarkably stable at or below -70°C. No significant potency losses could be detected, even after 66 months of storage. The 4 years shelf life for long term storage is thus acceptable. Stability was also satisfactory in short term storage (up to 4 weeks) at -18°C. The in-use stability at room temperature has been studied, justifying an overage to compensate the losses following thawing and in-use stability.

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

Not applicable

Overall conclusions on quality

The quality part of the dossier is clear and complete. No major objection was raised and essentially all other concerns are now clarified.

Part 3 – Safety

Safety documentation

According to the guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2006-rev2), as far as safety tests are concerned:

- For laboratory trials, the GLP requirements could be lifted, if justified.
- Field studies (if necessary) can cover safety and efficacy aspects in one trial. A more flexible approach may be taken in relation to compliance with Good Clinical Practice (GCP), provided sufficient justification.
- Literature may be used to support the safety and efficacy claim, provided these data were raised by testing the product, the application is made for. Bibliographic data should preferably originate from acknowledged scientific literature ideally from peer-reviewed journals. Exceptions must be justified.
- No max. dose/potency are required for laboratory studies, there are no passage level requirements
- No expert report needs to be provided (such a report was indeed not provided for this application).

The studies provided by the applicant were assessed taking into account these recommendations.

In particular, most reports on safety studies were prepared recently from old data. Most studies were not carried out in compliance with GLP/GCP, likely due to the fact that they are old. However, a statement accompanies all these studies, claiming they were performed according to a pre-defined protocol and relevant SOPs, and raw data are most of the time available. The validity of these studies (in relation to GLP requirements) was therefore accepted.

Laboratory tests

Safety of the administration of one dose

No specific study has been carried out. This is acceptable, as long as safety of the administration of an overdose is demonstrated. Also, the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2006-rev2) explicitly mentions that such test may not be carried out.

Safety of one administration of an overdose

In chickens: SPF chickens, 4 weeks-old, hybrid white leghorn, seronegative to *Mycoplasma synoviae* were used. The chickens from the test group were administered the vaccine by eye drop (each animal received 7.8 $\log_{10}$ CCU, which is very close to a 10 times overdose). A control group was left unvaccinated. The two groups were maintained in separate isolators throughout the experiment. Clinical examination was carried out daily. Body weight gain was determined. Twenty-one days post vaccination all chickens were euthanised and post mortems were carried out, with special emphasis on air sacs. Neither clinical signs linked to vaccination were observed, nor any lesion. Vaccination had no negative impact on body weight gain. It was therefore concluded that safety of the administration of an overdose in chickens of the minimum age recommended was demonstrated.

Another overdose study in chickens was carried out and provided in the responses to questions from CVMP: SPF chickens, 4 weeks-old, were used. The chickens from the test group were administered the vaccine by eye drop (each animal received 7.68-7.91 $\log_{10}$ CCU, which is very

close to a 10 times overdose). A control group was left unvaccinated. The two groups were maintained in separate isolators throughout the experiment. Clinical examination was carried out daily. Body weight gain was determined (between day 0 and day 21). Twenty-eight days post vaccination all chickens were euthanised and post mortems were carried out, with special emphasis on air sacs. Neither clinical signs linked to vaccination were observed, nor any lesion. Vaccination had no negative impact on body weight gain. This study therefore confirmed the safety of the administration of an overdose in chickens of the minimum age recommended.

Another study, provided for information only, further supports the safety of administration of an overdose of the vaccine in chickens.

In turkeys: limited published data (Noormohammadi et al, Avian Diseases, 2007) suggest that up to 5 x 10^7 CCU are safe for turkeys of 4 weeks of age: commercial turkeys, seronegative and free of *Mycoplasma synoviae* were administered the vaccine, either by eye-drop or aerosol. Two weeks later, they were euthanised and subjected to pathologic, microbiologic and histopathologic investigations: no gross lesions were found in air sacs and joints. No significant difference was found in the mean tracheal thickness compared to negative controls. *Mycoplasma synoviae* colonies were found in some of the vaccinated birds in the upper trachea.

Safety of the repeated administration of one dose

No study has been carried out to investigate the safety of the repeated administration of 1 dose. This is acceptable as the vaccination scheme does not include any booster.

Examination of reproductive performance

Administration of the vaccine is contra-indicated within 5 weeks before the onset of lay. Limited data suggest the vaccine is unlikely to have a negative impact on egg production.

Examination of immunological functions

No study on the examination of the potential impact of vaccination on the immunological functions has been conducted. Whereas some mycoplasma species are reported to have immunomodulatory effects and can suppress B or T lymphocyte proliferation and the production of cytokines, this has not been reported for *Mycoplasma synoviae*. Therefore, the absence of a specific study on the matter is acceptable.

Special requirements for live vaccines

- **Spread of the vaccine strain**

To animals of the target species

SPF chickens, 3 weeks-old, hybrid white leghorn, seronegative to *Mycoplasma synoviae* were used. Groups of chickens received the following treatment at day 0 by eyedrop:

Control Group: unvaccinated

Group 1: inoculated with MB strain of *M. synoviae* (mutant obtained by NTG mutagenesis)

Group 2: inoculated with MG strain of *M. synoviae* (mutant obtained by NTG mutagenesis)

Group 3: vaccinated using MS-H vaccine

Group 4: vaccinated using the parent *Mycoplasma synoviae* strain 80869/7NS

Three weeks after vaccination/inoculation, 2 birds of the control group were commingled with each of the other group. Swabs from choanal cleft were taken on unvaccinated controls 1 and 2 weeks after commingling with the respective groups. Swabs were cultured on Mycoplasma agar to detect

Mycoplasma synoviae. A representative number of samples (5-10%) were confirmed as *Mycoplasma synoviae* by immunofluorescent testing.

All controls remained culture negative 2 weeks after day 0, whereas at that time, all birds of group 3 were positive. One out of the 2 controls commingled with MS-H vaccine group became culture positive to *Mycoplasma synoviae* 1 week after commingling. The other control became positive 2 weeks after commingling. All other birds from group 3 were still positive at the end of the study. One out of the 2 controls put in contact with group 4 also became positive, while the other one died of causes unrelated to inoculation (as reported without further details). It was concluded that the vaccine strain was able to spread to in contact chickens.

Following comments of the CVMP, a new safety study to assess the spreading of the vaccine strain was carried out. The study included a higher number of animals per group.

Four week-old SPF chickens were administered MS-H vaccine by eyedrop at an overdose of $10^{7.68}$ to $10^{7.91}$ CCU/bird. Swabs were taken from the choanal cleft of the birds 14 days after administration of the vaccine to check for the presence of the MS-H strain.

On the same day, naïve SPF chickens were placed in the same isolator as the vaccinated birds. One week later the naïve birds had swabs taken of the choanal cleft (on Day 21 post vaccination) to check for the presence of spread of the MS-H strain. This procedure was repeated 2 weeks after co-mingling (on Day 28 post vaccination) to check for the presence of spread of the vaccine strain. All birds were euthanised on day 28 post vaccination and underwent a post mortem examination focused on the respiratory tract (trachea, air sacs).

The results showed that 100% of vaccinated birds had positive culture results 14 days after inoculation. The naïve in contact chickens had all positive culture results 14 days after commingling with vaccinated chickens while 20-30% (depending on the culture method) were negative 7 days after commingling. On post mortem examination, no lesion was detected in any chicken.

These results confirm that the vaccine strain is able to spread from vaccinated chickens to seronegative in contact chickens.

To non target animal species:

The safety of the spreading of the vaccine strain to non target animal species has not been investigated. *Mycoplasma synoviae* is pathogenic for turkeys, and has been isolated from various other avian species (guinea fowl, Japanese quail, pigeons, partridges, ducks, sparrow, geese and pheasants), although its pathogenicity for those species is not clear.

It is acknowledged that the vaccine strain might spread to other (avian) target species. If spreading occurs to these species, it is however unlikely that the vaccine strain might raise safety issues. Warnings on that issue have been inserted in the SPC.

- **Dissemination in the vaccinated animal**

Dissemination in the respiratory tract

In a preliminary study, a single dose of MS-H vaccine, with added blue-dye, was administered to six-week-old SPF chickens. They were euthanised respectively 5 and 20 minutes post vaccination. Five minutes post vaccination, staining was evidenced on the tongue, the larynx, as well as the conjunctiva, the palatine cleft and the crop. Twenty minutes post vaccination, the staining was more intense in the crop and the digestive tract.

In a second step, SPF chickens, 4 weeks-old, Hybrid white leghorn, seronegative to *Mycoplasma synoviae* were used.

Groups of chickens received the following treatment by (eye-drop) at day 0:

Group 1: 30 µl of MB

Group 2: 30 µl of MS-H vaccine (6.4 $\log_{10}$ CCU)

Group 3: 50 µl of parent *Mycoplasma synoviae* strain 80869/7NS (1.6 x 10^5 CCU)

Isolation of *Mycoplasma synoviae* was attempted from the following sites/tissues at various time intervals: palatine cleft, trachea, conjunctiva, larynx, infra-orbital sinuses and right and left air sacs.

The vaccine strain was shown to disseminate to the conjunctiva, the palatine cleft, the infra-orbitar sinus (IOS), the larynx, and the trachea, but apparently not to the air sacs. The rate of *Mycoplasma synoviae* isolation was higher for the parent strain, and there was a trend for the parent strain to be isolated more frequently in the lowest sections of the trachea.

The dissemination in the GI tract was not investigated further; this was adequately justified: although MS-H was shown to disseminate to the GI tract immediately after vaccination, it is unlikely to establish there, as wild *Mycoplasma synoviae* has never been found in faeces under field or experimental conditions, and because the vaccine strain is thermosensitive.

Dissemination into the reproductive tract and eggs

SPF, 16 weeks-old on day 0, ISA Brown layer birds, seronegative to *Mycoplasma synoviae* were used. The following groups were constituted:

Groups 1 and 2: 1 dose (6.3 $\log_{10}$ CCU) of MS-H vaccine by eye-drop at 19 weeks of age

Groups 3 and 4: challenge strain *Mycoplasma synoviae* 94011V-18 d (titre not described) onto the oviduct via the cloacal cavity and then IBV V1/71 (4.2 $\log_{10}$ EID50) intratracheally and *Mycoplasma synoviae* challenge strain by aerosol at 19 weeks of age (positive controls)

Group 5: Sterile MB by eye drop at 19 weeks of age (negative controls)

The birds from the different groups were maintained in separate isolators throughout the study.

Egg production was monitored throughout the study. Oviducts and eggs were monitored weekly from 1 to 4 weeks post inoculation for contaminations by *Mycoplasma synoviae*. Oviduct contamination was detected only in groups 3 and 4 (from 8.3 to 38.5% of oviducts contaminated depending on the time points). No contamination of the eggs was detected in any group. Groups 1 and 2 had similar egg production % compared to controls. Groups 3 and 4 had reduced egg production %.

Although it is recognised that the probability to detect the vaccine strain in the limited number of eggs sampled in this study was likely to be very low, the negative isolation results obtained for the vaccine group in this study (oviducts and eggs) coupled to the presence of a positive control group validating the detection of *Mycoplasma synoviae* from the oviduct, together with the ts nature of the vaccine strain, as well as the contra-indication to use the product within 6 weeks before the onset of lay allow to conclude that the probability of transmission of the vaccine strain to the eggs, if the vaccine is used as recommended, is negligible. Similarly, a negative impact on egg production is unlikely.

- **Reversion to virulence of attenuated vaccines**

An earlier study on reversion to virulence was undertaken but was not to VICH guidelines. A new reversion to virulence study was requested by CVMP and , the applicant has carried out a new reversion to virulence study, according to the VICH GL 41:

SPF chickens, 4 week-old were administered by eye drop the vaccine at a titre higher than the maximum release titre. The working seed was used rather than the master seed because of the fact that the stocks of the master seed are very limited. This explanation was acknowledged by the CVMP.

Six days after inoculation, the chickens were euthanised and their respiratory tract (mouth, nares and trachea) was swabbed to collect *Mycoplasma synoviae.*

The harvested material was used for subsequent inoculation of a further group of SPF chickens. These chickens were similarly held for 6 days to allow colonisation of the inoculated organisms, before euthanasia and recovery of the colonised organisms. This process was repeated for a total of five passages.

The amounts administered to the next passage birds were based on the approximate viable count estimated using qPCR, the volume available and the maximum volume that the birds' eyes could absorb. Viable counts were determined a posteriori, because of the time needed for M. synoviae to grow in vitro.

Birds at each passage level were observed for clinical signs of disease after inoculation, and underwent post-mortem examination with a particular focus on the respiratory tract.

At the time of inoculation of the final passage (P5), three further groups of SPF birds were inoculated with: a dose of MS-H Vaccine Working Seed (1xMaxRT dose); material harvested from negative control birds, which had received no treatment; and material harvested from the P1 birds. These groups were examined daily for clinical signs for 21 days and were then euthanised. Post mortem examination included specific scoring of any pathology present in the air sacs, and measurements of tracheal mucosal thickness.

MS could be isolated from each passage. Titre tended to decrease from P1 to P5.

No clinical sign was evidenced in any treated group, except a slight depression in 1 animal from P2 for a few hours post inoculation. No air sac or tracheal lesion was detected in any group in the final comparison (P1, P5, working seed and negative control). The tracheal mucosal thickness was not significantly different between groups. A number of isolates from P1 and P5 were tested for thermosensitivity and all were found to be thermosensitive.

The vaccine strain was therefore shown not to revert to virulence after 5 *in vivo* passages.

Concerning the loss of the thermosensitive phenotype among field isolates, the applicant provided further arguments. In particular, the following arguments were found sufficient to solve the reversion to virulence issue:

Even if the ts phenotype was lost by the vaccine strain in the field, it is true that the chickens administered the ts-isolates did not present air sac lesions, that are produced by the parent strain; this is most probably because the chemical agent used for mutagenesis is known to cause a high number of mutations, including in mycoplasma spp, as reported by the applicant. No safety problem was evidenced in the field after years of use of the product. This, in addition to the new reversion to virulence study, allow to conclude that the risk for reversion to virulence is likely negligible.

- **Biological properties of the vaccine strain**

The ts phenotype is one important characteristic of the vaccine strain, responsible, at least partly, for its attenuation.

- **Recombination or genomic re-assortment of the strains**

It is argued that *Mycoplasma synoviae* has a single haploid chromosome and does not contain plasmids and therefore recombination cannot occur. *Mycoplasma synoviae* has never been able to be made competent experimentally, thus transformation with exogenous genetic material is extremely unlikely (G. Browning pers. Comm. to the applicant). Absence of integrative conjugative elements, which have been associated with other mycoplasmas, further indicates that this organism will not undergo recombination in the environment.

The literature suggests that although there may have been genetic transfer between *M. synoviae* and *M. gallisepticum* this may have occurred over millennia. It was finally concluded that the risk of recombination or genomic re-assortment between the vaccine strain and other mycoplasma spp. was negligible.

Study of residues

No specific study of residues has been carried out. The following arguments were presented: MS-H is a live vaccine with no adjuvant or preservatives. The active ingredient is contained within a simple medium which does not contain any ingredients that would require residue studies.

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The components of the excipients listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this veterinary medicinal product. The medium also contains traces of phenol red resulting from the use of the substance in the fermentation process as a pH indicator. While the substance is used in the manufacturing process it is not an intended component of the final product and will be present at trace levels only. Therefore, in line with the CVMP Revised position paper on the definition of substances capable of pharmacological action this substance is considered as not falling within the scope of the MRL Regulation.

It was also noted that *Mycoplasma synoviae* is not a zoonotic agent and therefore does not represent a consumer safety concern.

The withdrawal period has been set to zero days.

Interactions

A limited study has been provided, the results of which suggest no evidence of additional adverse reactions when MS-H vaccine was used in combination with other commonly used avian vaccines. However, due to a lack of data on other vaccines interactions and in particular regarding efficacy, absence of interactions with other vaccines is not claimed in the SPC. The SPC mentions the appropriate standard sentences on that issue.

Field studies

One field study carried out in 3 different sites in Australia, covering both safety and efficacy aspects, was provided.

Site 1: commercial broiler breeder farm, with history of *Mycoplasma synoviae* disease for 3 years, both in the breeders and the progeny, consisting of single-age production farm, composed of 10 houses with 4000 birds per house.

Site 2: commercial layer-breeder farm, with history of seroconversion to *Mycoplasma synoviae* in parents and clinical disease in the progeny, consisting of a single age production farm and composed of 1 house with 4000 birds

Site 3: commercial broiler breeder farm consisting of multiple age production complex. A single-age farm of 4 houses, housing 32 000 birds, was used in this study. This farm was 300 m away from a similar sized production farm. Breeders were generally exposed to *Mycoplasma synoviae* between 18 and 20 weeks of age. Vertical transmission was also commonly reported in the progeny.

Vaccination, using MS-H vaccine, was carried out as follows (presumably by eye-drop):

Site 1: all broiler breeders were vaccinated at 5 weeks of age and moved on the production site at 20 weeks of age.

Site 2: all layer breeders were vaccinated at 18 weeks of age, on arrival at the production site.

Site 3: Two flocks of broiler breeders were vaccinated at 16 weeks of age and moved to the production site at 19 weeks of age. The remaining two flocks were unvaccinated.

Swabs (choanal cleft) were taken from control (where included) and vaccinated birds periodically to assess lateral transmission and/or persistence of the vaccine strain (by culture). Isolates were examined by RFLP to determine the strain of *Mycoplasma synoviae* (vaccine or wild). Fifty different isolates of *Mycoplasma synoviae* taken from vaccinated birds, all of which had been confirmed as the vaccine strain by RFLP, were incubated to determine the ts phenotype.

Samples of yolk sacs of the embryos (from sites 1 and 3) were taken for culture to assess the vertical transmission of *Mycoplasma synoviae*.

Broiler progeny from site 1 were also tested for serum antibody by HI test at 56 days of age to assess the vertical transmission of *Mycoplasma synoviae*.

Concerning the safety, on one hand, isolation and serological data further suggest that the vaccine strain is not vertically transmitted (however, embryo samples for isolation and blood samples from progeny broilers were taken long after vaccine administration - respectively 55 weeks and at least 15 weeks thereafter - which clearly might have an impact on the occurrence of vertical transmissionConcerning the lateral spread of the vaccine strain (site 3), results suggest that the vaccine strain is able to disseminate to nearby flocks. However, in the absence of checking of the microbiological status of the animals before vaccination, the results should be interpreted with caution.

The fact that the vaccine strain may persist in the respiratory tract of chickens for up to 55 weeks post vaccination has been inserted in the SPC.

User safety

Mycoplasma synoviae is not a zoonotic agent. Therefore, no specific risk for the user is expected should any person be accidentally exposed to the active ingredient.

Inactive components of the product: the product does not contain any adjuvant. The inactive components present in the vaccine are only media components from the fermentation of the vaccine strain. None of these constituents are expected to cause any toxicity issue in any person accidentally exposed to the product. The pH of the product is between 6.7 and 7.1, which is close to neutral. Therefore, no irritation is expected to occur if skin or eyes is exposed to the product.

The fact that the bottle are made of plastic minimises the risk that the bottle breaks.

Risks for the user of the product are limited to skin (handling of frozen bottles) and eye (rapidly thawing small pieces of ice becoming a projectile) injuries which might occur by manipulation of the frozen bottle. A specific warning is included in the SPC which advises the following:

To avoid skin and eye injuries which may occur by manipulating the frozen bottle, protective gloves and safety glasses should be worn.

Another, acceptable, warning is included in the SPC, as an additional precaution:

"In case of accidental spillage on skin wash immediately with disinfectant. If vaccine is accidentally splashed into the operator's eye, the eyes and face should be thoroughly washed with water to avoid any potential reaction to culture medium constituents".

Environmental risk assessment

The scheme recommended in the Guideline on ERA for IVMPs (EMEA/CVMP/074/95) has been generally followed. Taking into account the clarifications and new data provided by the applicant (spreading, recombination, reversion to virulence and stability of the thermosensitive phenotype), the CVMP concluded that administration of the vaccine does not cause a risk (or only a negligible risk) to the environment.

Overall conclusion on safety

No safety test on the administration of 1 dose has been carried out in chickens. This is acceptable as covered by an overdose study. The safety of the administration of a titre of vaccine close to a 10-times overdose is considered demonstrated in SPF chickens of 4 weeks of age: no clinical signs and no lesions were observed after administration of the vaccine. There were no negative impacts on the body weight gain. No study has been carried out to investigate the safety of the repeated administration of 1 dose. This is acceptable as the vaccination scheme does not include any booster.

Administration of the vaccine is contra-indicated within 5 weeks before the onset of lay. Limited data suggest the vaccine is unlikely to have a negative impact on egg production.

No study on the examination of the potential impact of vaccination on the immunological functions has been conducted. Whereas some mycoplasma species are reported to have immunomodulatory effects and can suppress B or T lymphocyte proliferation and the production of cytokines, this has not been reported for *Mycoplasma synoviae*. Therefore, the absence of a specific study on the matter is acceptable.

The vaccine strain is able to spread from vaccinated chickens to seronegative in contact chickens.

A dissemination study carried out in the target species has shown that the vaccine strain disseminates to the conjunctiva, the palatine cleft, the IOS, the larynx, and the trachea, but apparently not to the air sacs.

Another dissemination study supports the absence of vertical transmission of the vaccine strain.

The reversion to virulence study suffered from major deficiencies and a new study, has been carried out following the recommendations of the VICH GL 41 (EMEA/CVMP/VICH/1052/2004). In that study, the vaccine strain was shown not to revert to virulence after 5 *in vivo* passages: no clinical sign nor any tracheal or air sac lesions were evidenced in chickens administered the 5th passage. Also, there were no differences in tracheal mucosal thickness between the groups (administered either the 1st passage, the 5th passage, the working seed or negative controls).

Horizontal gene transfer may have been occurred between *Mycoplasma synoviae* and *Mycoplasma gallisepticum*, and a a review of the literature has been done on the matter. It was concluded that the risk of recombination or genomic re-assortment between the vaccine strain and other mycoplasma spp was negligible.

The CVMP concluded that based on the information provided the product has an acceptable level of safety.

Risks for the user of the product are limited to skin and eyes injuries which might occur by manipulation of the frozen bottle. The CVMP concluded that these are appropriately addressed through specific warnings in the product literature.

No claim of compatibility with other vaccines is made.

The CVMP concluded that the active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The components of the excipients listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this veterinary medicinal product.

The withdrawal period has consequently been set to zero days.

In the field studies carried out in Australia, the reversion to a ts-phenotype for 9 isolates out of 50 was of great concern. The suitability of the vaccine strain to be used as a vaccine strain was seriously questioned and has consequently been thoroughly justified.

Environmental risk assessment has been updated after clarifications and new data provided by the applicant on the following issues: spreading, recombination, reversion to virulence and stability of the ts phenotype. The CVMP concluded that administration of the vaccine does not cause a risk (or only a negligible risk) to the environment.

Part 4 – Efficacy

Introduction and general requirements

The parent of strain MS-H was isolated from the choanal cleft (palatine fissure) of a commercial layer hen in Australia (Morrow 1990a). It was subject to chemical mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (NTG). The most recognisable mark of mutagenesis of the vaccine strain is its temperature-sensitive phenotype. The choice of the vaccine strain has been sufficiently justified. By definition there is only one serotype of *Mycoplasma synoviae*. Documentation has been provided to document the cross-reaction towards European *Mycoplasma synoviae* strains. The majority of the challenge experiments used a *Mycoplasma synoviae* strain isolated in Australia, but 1 published article also deals with the demonstration of efficacy against a European strain (Feberwee et al, 2009).

According to the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2006-rev2), as far as efficacy tests are concerned:

- No min. dose/potency are required for laboratory studies, there are no passage level requirements
- Literature may be used to support the safety and efficacy claim, provided these data were raised by testing the product, the application is made for. Bibliographic data should preferably originate from acknowledged scientific literature ideally from peer-reviewed journals. Exceptions must be justified.
- Should adequate documentation not exist in the literature, the efficacy of the product should be demonstrated in appropriately designed studies. The type and number of studies to be conducted will depend on the deficiencies in available data.
- Field studies (if necessary) can cover safety and efficacy aspects in one trial. A more flexible approach may be taken in relation to compliance with Good Clinical Practice (GCP), provided sufficient justification.
- It is recognised that existing studies may not satisfy current GCP requirements. Such studies should be considered acceptable if the design is appropriate to the stated objective of the study.
- The applicant should test for treatment differences using appropriate statistical methodology. It should be possible in all cases to demonstrate a benefit of treatment (either relative to a control or, where appropriate, relative to pre-treatment data) that is statistically significant. However, the practical limitations of data collection for a minor market product will be taken into consideration.
- No expert report needs to be provided (such report was indeed not provided for this application).

The studies provided by the applicant were assessed taking into account these recommendations.

Laboratory trials

Establishment of a Challenge Model.

The following elements were considered for its development:

Reproducing air sac lesions was the main criteria desired by the applicant.

Developing a valid challenge model using only *Mycoplasma synoviae* as infectious agent is difficult: it was therefore chosen to use a combined challenge *Mycoplasma synoviae* + IBV. Such combined challenge was already reported in the literature and has resulted in a successful reproduction in previous experiments (Kleven et al, 1972, Yoder et al, 1977).

An heterologous *Mycoplasma synoviae* strain (different from the vaccine strain) was selected.

A non-nephrotoxic IBV strain was used.

Seven week-old HWL SPF chickens, seronegative to *Mycoplasma synoviae*, were used.

At 7 weeks of age, the different groups were challenged as follows:

Group 1: Sterile MB by aerosol

Group 2: undiluted *Mycoplasma synoviae* by aerosol

Group 3: IBV IT

Group 4: IBV IT + undiluted *Mycoplasma synoviae* by aerosol

Group 5: IBV IT + *Mycoplasma synoviae* diluted 1:10 by aerosol

Group 6: IBV IT + *Mycoplasma synoviae* diluted 1:100 by aerosol

Group 7: IBV IT + *Mycoplasma synoviae* diluted 1:1000 by aerosol

Two weeks later, birds were euthanised. AS lesions were assessed grossly for severity on a scale of 0 - 3 (as described by Nunoya et al, 1987), with each of the right and left anterior and posterior thoracic and abdominal AS scored individually. A cumulative AS lesion score was then determined for each bird by adding each of these scores (Markham et al, 1998). AS lesion incidence in each group was calculated as the % of all AS in each group with lesions (Markham et al, 1998).

A dose response was observed on the development of AS lesions. The combined challenge IBV + *Mycoplasma synoviae* administered either at a dose undiluted or diluted a: resulted in the most severe AS lesions (significantly more severe than the group challenged with IBV only) and the highest incidence of AS lesions. Based on the numerically higher incidence and severity of lesions, the combined challenge IBV + undiluted *Mycoplasma synoviae* was adequately selected.

Although the assessment of the lesions seems largely subjective, this evaluation is close to the one recommended by EP for (inactivated) vaccines against *Mycoplasma gallisepticum*. Given gross lesions induced by *Mycoplasma synoviae* in the respiratory tract are similar to those caused by a *Mycoplasma gallisepticum* infection (Bradbury and Morrow, 2008), this is acceptable.

For all the "pivotal" efficacy studies, the same combined challenge model was applied.

However, given the challenge strain was only able to produce AS lesions, and not, for example synovitis, and that a combined IBV-MS challenge was used, only a claim of reduction of AS lesions due to secondary infections caused by *Mycoplasma synoviae* could be considered for this vaccine. Also, clarifications are still needed on the effect on AS lesions that can be expected from vaccination in case of co-infection by European IBV strain.

Determination of the vaccine dose

Five week-old HWL SPF chickens, seronegative to *Mycoplasma synoviae*, were randomly allocated to 8 groups. Groups 1 and 2 were controls (administered sterile MB by eye-drop). Groups 3 to 8 were administered various doses of MS-H vaccine by eye-drop(

At 6 weeks post vaccination, birds from groups 2 to 8 were challenged as described above. Birds from group 1 were kept as true negative controls. Two weeks post challenge, lesions (air sacs +

tracheal thickness) and presence of *Mycoplasma synoviae* within trachea and left abdominal AS were investigated. Serological response (RSA) was monitored before and after challenge.

The minimum protective dose was determined. There was a clear difference in the results (significant differences for serological response, air sac and tracheal lesions) obtained using the minimum dose and the next lowest In contrast, there was no obvious benefit in increasing the dose above the minimum one. In this study, tracheal thickness was also envisaged, in addition to the parameters described in the study on the establishment of a challenge model. As recognised by Jones et al (2006a), although such criteria are used in the frame of experimental challenges with *Mycoplasma gallisepticum*, the relevance of such criteria in the context of the combined challenge *Mycoplasma synoviae* + IBV can not be completely ascertained, because of the absence of a control group challenged only with IBV. Therefore, as far as vaccine efficacy is concerned, these data are of limited interest. In all the challenged groups, the culture scores were generally higher in the trachea than in the AS. Although there was a trend in reduction of the culture scores between groups 5-8 compared to groups 2-4, no significant difference was evident.

Onset of protection

Five week-old hybrid white leghorn (HWL) SPF chickens, seronegative to *Mycoplasma synoviae*, were randomly allocated to different groups. At 5 weeks of age, birds from the vaccine group were administered 120 µl of MS-H vaccine by eye-drop (6.8 $\log_{10}$/bird, to be confirmed) and birds from the control group were administered 50 µl of sterile MB. At 1, 2, 3, 4, 5 and 6 weeks post vaccination, a number of birds from each group were challenged as described above. Two weeks post challenge, lesions (air sacs + tracheal thickness) were investigated. Serological response (ELISA and RSA) was monitored before and after challenge. From 3 weeks post vaccination onward, a serological response was detected in the majority of birds, which was not the case at 1 and 2 weeks pv. No significant difference for air sac lesion severity or incidence was observed between control group and vaccinated animals challenged at either 1, 2 or 3 weeks post vaccination. From 4 weeks post vaccination onward, there was a significant difference for both air sac lesion severity and air sac lesion incidence. The tracheal mucosal thickness was significantly lower in the vaccinated groups when challenged from 4 weeks onward post vaccination, whereas no significant difference was observed when vaccinated animals were challenged before 3 weeks post vaccination.

An onset of protection of 4 weeks is therefore demonstrated. However, given the challenge model only induced air sac and tracheal lesions (and not synovitis), that the relevance of the tracheal lesions in the combined challenge is debatable and that reduction of tracheal lesions was not consistently achieved, the CVMP decided to restrict the claim as follows: "For active immunisation of future broiler breeder chickens, future layer breeder chickens and future layer chickens to reduce air sac lesions and reduce the number of eggs with abnormal shell formation caused by *Mycoplasma synoviae.*

Additionally the CVMP concluded that for this vaccine it was important to add information under special warnings which clearly states that there was no effect demonstrated on respiratory clinical signs, systemic colonisation and vertical transmission.

Influence of maternal antibody on the efficacy of the vaccine

No study has been performed to address this issue. Limited published data (MacOwan et al, 1984) suggest that maternally derived antibodies to *Mycoplasma synoviae* would have disappeared at the time of vaccination (5 weeks of age). Also, the use of the vaccine is only recommended in seronegative flocks. iIt can therefore be concluded that the published data on maternally derived antibodies are sufficient to demonstrate no influence on vaccination.

Duration of immunity

Five week-old HWL SPF chickens, seronegative to *Mycoplasma synoviae*, were randomly allocated to different groups. At 5 weeks of age, the birds from the vaccinated group were administered 4 drops of MS-H vaccine by eye drop (5.7 $\log_{10}$ CCU/dose, total dose per chicken: 6.3 $\log_{10}$ CCU). Birds from the control group were given 30 µl sterile MB. At 15 and 40 weeks post vaccination, a number of birds per group were removed and challenged as described above. Two weeks post challenge, birds were euthanised and lesions (air sacs + tracheal thickness) were examined. Isolation of *Mycoplasma synoviae* was attempted from the following tissues: trachea (UT, MT, LT), air sacs (on every animal at post mortem); brain, liver, kidney, spleen, oviduct (only from animals challenged at 40 weeks post vaccination). Serological response (RSA and ELISA) was also investigated.

Vaccination induced an increase of the serological response. This study supports a duration of protection of 40 weeks post vaccination, because at both timepoints (15 and 40 weeks), vaccination significantly reduced the incidence and severity of air sac lesions compared to controls. The effect of vaccination on tracheal inflammation induced by the challenge was not consistent (not systematically reduced at 15 weeks post vaccination) (see also comments elsewhere on the relevance of this criteria). Significantly lower culture scores were observed for all tracheal tissues and and air sacs of the MS-H vaccinates compared to the non-vaccinated control groups in the birds challenged at 15 and 40 weeks post vaccinaation. At 42 weeks, *Mycoplasma synoviae* was isolated from the brain of 1 control, the spleen of another 1 control and the kidney of another 5 controls (7 controls affected on the whole out of 9). *Mycoplasma synoviae* was confirmed to be the challenge strain by PCR and DNA restriction assay. No *Mycoplasma synoviae* was isolated from any liver or oviduct from any bird, nor from any brain, kidney and spleen from the MS-H vaccinated group. This study therefore suggests that vaccination might reduce the risk for respiratory tract colonization and further systemic infection but no significant reduction was seen

Additional studies

Another laboratory efficacy study (MS GD R0913) has been provided by the applicant, describing the effect of vaccination with MS-H vaccine against a recently described pathology, i.e. eggshell apex abnormalities. An association between infection with *Mycoplasma synoviae* and eggshell apex abnormalities has been recently reported (Feberwee et al2009).

Twelve week-old SPF white layer hens, seronegative and free of *Mycoplasma synoviae* were used. They were divided into weight classes and allocated in 4 groups so that the average weight was not significantly different between groups. At 14 weeks of age, they were administered by eye drop as follows:

Group 1: 23 µl of ME medium (not vaccinated not challenged, NVNC).

Group 2: 23 µl of MS-H vaccine, commercial batch MSH 072991A, (vaccinated not challenged, VNC).

Group 3: 23 µl of ME medium (not vaccinated challenged, NVC)

Group 4: 23 µl of MS-H vaccine (vaccinated challenged, VC).

At 18 weeks of age: All groups were given Infectious Bronchitis variant (IBV) D1466 challenge (intra-tracheal (IT) + intramuscular (IM)) followed 5 days later, for groups 3 and 4, by IT inoculation of a *Mycoplasma synoviae* strain isolated in The Netherlands from the oviduct of a bird. Serological response to *Mycoplasma synoviae* and IBV was monitored. From 19 weeks to 30 weeks old, egg production, number of eggs with eggshell apex abormalities (EAA) and eggshell strength were recorded. Birds were euthanised at 30 weeks old. Tracheal swabs were taken and CFU

equivalents were determined by PCR; Culture of *Mycoplasma synoviae* was also attempted from oviducts and isolates were identified by PCR.

Only birds vaccinated and/or challenged by *Mycoplasma synoviae* had antibodies to *Mycoplasma synoviae*. All birds were seropositive to IBV D1466 at the end of the experiment. The challenge could be considered validated by comparing the results of not vaccinated (NVC) and not vaccinated not challenged (NVNC) groups: it induced *Mycoplasma synoviae* contamination of the trachea and of the oviduct as well as a significant decrease of egg production and a significant increase of proportion of EAA eggs. The study showed a positive effect of vaccination on the proportion of EAA produced after challenge. However, vaccination was not shown to allow a significant reduction of the tracheal or oviduct contamination by the *Mycoplasma synoviae* challenge strain (vaccinated (VC) group compared to NVC group). Although the vaccinated group had a higher amount of egg produced per chicken compared to the NVC group, no significant difference was achieved.

A study (MSH 100631) was provided to support the relevance of the vaccine strain, isolated in Australia, to protect against European strains of *Mycoplasma synoviae*: a collection of European *Mycoplasma synoviae* strains were obtained. Those strains, confirmed as *Mycoplasma synoviae* by IF, are classified into different groups according to the sequence of the *vlh*A gene (encoding the MSPB protein). All the groups were represented, except groups 3 and 7. Vaccine strain and reference type strain of *Mycoplasma synoviae*, WVU-1853 were also tested. The different strains were grown in modified MB, whole cell proteins were extracted and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis SDS-PAGE and identical gels stained with Coomassie brilliant blue or Western transferred to poly-vinyldifluoride membranes. The sera used in the immunoblot corresponded to pools obtained from chickens administered MS-H vaccine by eyedrop. Negative sera (from control SPF chickens) were also used.

The whole cell protein profile (number and size of the proteins) was consistent throughout all strains tested. The post vaccination positive control sera reacted in Western blot producing several major bands in all *Mycoplasma synoviae* strains tested. This study generally supports the relevance of the vaccine strain for the EU situation. The fact that the vaccine is able to confer protection against a European strain has also been shown by challenge (Feberwee et al, 2009), which is more indicative than the above mentioned study.

Additionally, a study (MSH R0911) was carried out to examine the nature of the antibody response following administration of MS-H vaccine: Seven week-old SPF HWL chickens, seronegative to *Mycoplasma synoviae* were randomly allocated in different groups. Birds from the vaccinated group were administered 30 µl of MS-H vaccine by eye-drop . A control group was left unvaccinated. A number of birds were euthanised for sample collection at different time points from 24 hours to 5 weeks after inoculation: serum, tracheal washings, lacrimal fluid, nasal washings, bile and Harderian gland. All these samples were tested by ELISA using the *Mycoplasma synoviae* surface antigen, MSPB to determine the respective levels of IgM, IgG and IgA.

After vaccination, IgM was originally detected in the Harderian Gland and also in the lacrimal fluid. IgG in serum persisted for at least 5 weeks in most birds, while IgM persisted for at least 5 weeks in a smaller number of birds. This study showed that vaccination induces both local and systemic antibody responses against MSPB, a major membrane antigen of *Mycoplasma synoviae*, described as a haemagglutinin. However, the extent to which those antibody responses are correlated to protection is unknown.

Field trials

One field study carried out in 3 different sites in Australia, covering both safety and efficacy aspects, was provided.

Site 1: commercial broiler breeder farm, with history of *Mycoplasma synoviae* disease for 3 years, both in the breeders and the progeny, consisting of single-age production farm, composed of 10 houses with 4,000 birds per house.

Site 2: commercial layer-breeder farm, with history of seroconversion to *Mycoplasma synoviae* in parents and clinical disease in the progeny, consisting of a single age production farm and composed of 1 house with 4,000 birds

Site 3: commercial broiler breeder farm consisting of multiple age production complex. A single-age farm of 4 houses, housing 32 000 birds, was used in this study. This farm was 300 m away from a similar sized production farm. Breeders were generally exposed to *Mycoplasma synoviae* between 18 and 20 weeks of age. Vertical transmission was also commonly reported in the progeny.

Vaccination, using MS-H vaccine, was carried out as follows (presumably by eye-drop):

Site 1: all broiler breeders were vaccinated at 5 weeks of age and moved on the production site at 20 weeks of age.

Site 2: all layer breeders were vaccinated at 18 weeks of age, on arrival at the production site.

Site 3: Two flocks of broiler breeders were vaccinated at 16 weeks of age and moved to the production site at 19 weeks of age. The remaining two flocks were unvaccinated.

Swabs (choanal cleft) were taken from control (where included) and vaccinated birds periodically to assess lateral transmission and/or persistence of the vaccine strain (by culture). Isolates were examined by RFLP to determine the strain of *Mycoplasma synoviae* (vaccine or wild). Fifty different isolates of *Mycoplasma synoviae* taken from vaccinated birds, all of which had been confirmed as the vaccine strain by RFLP, were incubated at 33 and 39.5°C to determine the ts phenotype.

Samples of yolk sacs of the embryos (from sites 1 and 3) were taken for culture to assess the vertical transmission of *Mycoplasma synoviae*.

Broiler progeny from site 1 were also tested for serum antibody by HI test at 56 days of age to assess the vertical transmission of *Mycoplasma synoviae*.

As far as efficacy is concerned, very few conclusions can be drawn from this study, as production data are not available, and because of shortcomings in the design of the study (such as absence of controls except in site 3, introduction of other management practices along with vaccination, absence of description of the serological and microbiological status of the flocks before vaccination). The data suggest that the vaccine induces a serological response under field conditions and the vaccine strain is able to persist in the respiratory tract of vaccinated birds for up to 55 weeks, although the persistence was not invariably detected in all vaccinated birds of the flock(s). Also, from the limited investigations carried out to detect MS strains in the site 3, it seems that the vaccine strain was progressively replaced by a MS field strain that was not shown to circulate before the onset of the immunity afforded by vaccination.

Summary of efficacy data

According to the proposed SPC, the vaccine is recommended:

"For active immunisation of future broiler breeders, future layer breeders and future layer birds to reduce air sac lesions caused by *Mycoplasma synoviae*.

Onset of immunity 4 weeks after vaccination.

The duration of immunity has been demonstrated to be 40 weeks post vaccination.

Evidence from the field shows vaccination can reduce vertical transmission of *M. synoviae* to progeny. In addition vaccination of flocks can increase egg production and reduce the number of eggs with abnormal shell formation caused by M. synoviae".

Vaccination (1 dose of 30µl by eyedrop) should be carried out on *Mycoplasma synoviae* -free birds of at least 4 weeks of age and at least 4 weeks before expected exposure to virulent *Mycoplasma synoviae*. The vaccine should not be used within 5 weeks of lay. Only flocks with no antibodies to *Mycoplasma synoviae* should be vaccinated.

These indications have been questioned. As explained by Whithear (1996), in general, potential objectives of the vaccination against *Mycoplasma synoviae* are:

-Reduction/prevention of respiratory clinical signs
-Reduction/prevention of air sac lesions
-Reduction/prevention of egg production losses
-Reduction/prevention of egg transmission.

More precisely, for breeding stock, the goal may be to reduce egg transmission (to have Mycoplasma free progeny) and to reduce contamination of the respiratory tract (to reduce the risk of transmission to other flocks (within or between farms). For breeder/layer chickens, a valid goal may be to reduce the egg production losses.

The following table summarises the results of the laboratory efficacy studies:

Study/efficacy parameters	**AS lesions**	**Tracheal lesions**	**Colonization of the respiratory tract**	**Reduction of Egg Apical Abnormalities**	**Egg production losses**	**Vertical transmission**	**Clinical signs**
Challenge model (MSH R0920)	Challenge reproduces AS lesions	ND*	ND	ND	ND	ND	ND
Dose determination (MSH R0908)	Reduction	Reduction	NO reduction# (trachea and AS)	ND	ND	ND	ND
OOI (MSH R0909)	Reduction	Reduction	ND	ND	ND	ND	ND
DOI (MSH R0910)	Reduction	NO reduction at 15 weeks pv Reduction at 40 weeks pv	Reduction at 15 and 40 weeks pv (trachea and AS)	ND	Investigated, but results not interpretable	NO effect demonstrated (no contamination of the oviduct in any challenged animal, including controls)	ND
Feberwee et al, 2009 (MS GD R0913)	ND	ND	NO reduction (trachea)	Reduction of EAA at 4 weeks pv	NO reduction	NO reduction (oviduct contamination)	ND

*ND: not determined/investigated within the study.

§reduction means significant reduction

#NO reduction means no significant reduction

The choice of the challenge model, which is a combined MS/IBV has been justified. The latter is able to produce AS lesions.

It is clear that the vaccine has consistently been shown to reduce air sac lesions caused by the combined challenge. Such an indication is therefore demonstrated, with OOI and DOI of respectively 4 and 40 weeks. Given the respective studies were carried out in chickens from 5 weeks of age, the minimum age for vaccination has been fixed accordingly.

The relevance of such claim had to be thoroughly justified taking among others into account the subcategory of target species (future breeders and future layers). In the challenge studies in which (likely more interesting) other efficacy parameters were investigated (such as respiratory tract and oviduct colonisation, or egg production), the results were either inconsistent throughout the studies (respiratory tract colonization) or no effect was demonstrated (oviduct contamination and egg production). Finally, taking into account that all the efficacy data were generated using a combined challenge IBV-MS, the applicant had to justify the efficacy that might be expected from the use of the vaccine in face of a (single) infection caused by *Mycoplasma synoviae*, and to propose a rewording of the indications (eg, the mention "due to *Mycoplasma synoviae* infection"), if appropriate.

The applicant agreed to restrict the (main) claim to reduction of air sac lesions caused by *Mycoplasma synoviae*. In addition, the applicant proposed additional secondary claims, as follows:

'Evidence from the field shows vaccination can reduce vertical transmission of M. synoviae to progeny. In addition vaccination of flocks can increase egg production and reduce the number of eggs with abnormal shell formation caused by *M. synoviae*.'

The applicant was requested to provide further justification of the interest of having a vaccine with a single claim of reduction of air sac lesions in future broiler breeders, future layer breeders and future layer chickens. According to the applicant, if reduction of lesions is ensured through vaccination against a challenge *M. synoviae* strain, this indicates that the challenge strain is less able to colonise the chickens, which in turn means that clinical signs (such as respiratory disease or synovitis) will be less important and that vertical transmission will also be reduced. While this could be true, no formal demonstration of such correlation has been provided by the applicant: in particular, not any laboratory study provided in the dossier allows to answer the question: indeed, the challenge model was shown to produce consistently air sac lesions, but does not reproduce clinical signs. In the only laboratory challenge study in which vertical transmission and egg production losses was evidenced, the presence and severity of air sac lesions was not investigated.

Regarding the efficacy that might be expected from the use of the vaccine in face of a single infection caused by *M. synoviae* no data are available. The reasoning that the vaccine will have a better effect on single MS infection is therefore speculative. However it is recognised that MS infection alone in a field situation is of low probability.

The applicant further justified another claim for the vaccine.

Claim for reduction of vertical transmission: To support this claim, the Applicant refers to the field study carried out in Australia (which was already assessed in the original dossier). This field study largely relies on historical controls and the applicant itself agreed that these data were not pivotal and of limited interest. In contrast, in the only laboratory study (MSGD R0913) in which the challenge strain was shown to contaminate the oviduct, there was no difference between the vaccinated and controls. Therefore the CVMP considered this claim to be not acceptable.

Claim for reduction of the number of eggs with egg shell abnormalities: The applicant has provided 1 challenge study, allowing to support this claim, with an associated OOI of 4 weeks (hens were vaccinated at 14 weeks old and challenged 4 weeks later), but no DOI. The CVMP concluded that this claim is acceptable with the appropriate wording regarding the lack of a DOI in the SPC.

Claim for increased egg production: For that claim, the applicant provided the results of a field study. This study, carried out in Japan, seems to show that vaccination has allowed to increase the egg production compared to historical controls. To the opinion of the CVMP, this study can be considered at best as supportive for the following set of reasons:

It was carried out in Japan,

It uses historical controls and not contemporary controls,

The study does not seem having been carried out according to a pre-established protocol. It is rather a report written a posteriori,

Evidence for a MS challenge is lacking

In addition and most importantly, in the laboratory study (carried out using a strain isolated in NL), where the challenge strain induced egg production losses, no significant difference was achieved between vaccinated and control group.

Therefore the CVMP considered this claim to be not acceptable.

Sufficient information has been provided for the relevance of the vaccine strain. While it has been isolated in Australia, it was shown to confer (some) protection against a challenge using a European *Mycoplasma synoviae* strain. Also, sera from vaccinated chickens cross –reacted against a panel of genetically distinct *Mycoplasma synoviae* strains isolated in Europe.

Information relative to the likely efficacy of the vaccine in presence of MDA is deemed sufficient, as MDA to *Mycoplasma synoviae* have been reported to become undetectable within 12 days after hatch. Given the vaccine has to be administered from 5 weeks of age this is acceptable.

No compatibility with other vaccines is claimed.

Field studies did not bring additional efficacy information.

During the oral hearing the applicant stated that in many countries around the world, antimicrobial use against mycoplasmas has fallen to very low levels once M. synoviae is well controlled using the live vaccine. The applicant has been further requested to provide additional information on the potential benefit that vaccination against *M. Synoviae* could result in a decreased use of antimicrobials. Information was supported by 4 case reports in Germany, South Africa, Mexico and USA. Following vaccination the use of antimicrobials was reduced or stopped and it was driven by management considerations to not use antimicrobials or to use products without activity against mycoplasma. There were no reports of outbreaks requiring the use of antimicrobials active against mycoplasma, except if the vaccination program was discontinued.

Overall conclusion on efficacy

Overall the CVMP concluded that only the claim for active immunisation of future broiler breeder chickens, future layer breeder chickens and future layer chickens to reduce air sac lesions and reduce the number of eggs with abnormal shell formation caused by *Mycoplasma synoviae* has been satisfactorily justified.

Part 5 – Benefit-risk Assessment

Benefit assessment

Direct benefits

The vaccine induces active immunisation of chickens from 5 weeks of age (layer replacement chickens, future broiler breeder chickens, layer chickens) and has been shown to reduce air sac lesions caused by a combined IBV-*Mycoplasma synoviae* infection. Onset and duration of immunity of respectively 4 and 40 weeks after vaccination have been demonstrated. The relevance of the vaccine strain has been shown for EU.

One laboratory study showed a positive effect of vaccination on the proportion of EAA eggs produced after challenge.

It is noted that the potential objectives of the vaccination against *Mycoplasma synoviae*, *i.e.* prevention or reduction of respiratory clinical signs, of systemic colonisation, vertical transmission and of egg production losses, have not been demonstrated.

Indirect benefits

Theoretically there is a potential for reduction of use of antimicrobials.

Additional benefits

None have been shown.

Risk assessment

The risk of recombination or genomic re-assortment between the vaccine strain and other mycoplasma spp was negligible.

Risks for the user of the product are limited to skin and eyes injuries which might occur by manipulation of the frozen bottle and have been appropriately addressed in the SPC. The administration of the vaccine does not cause a risk (or only a negligible risk) to the environment.

Vaccination might interfere with serological survey programmes of *Mycoplasma gallisepticum* and this risk has been adequately communicated in the SPC.

There is a risk of spread of the vaccine strain from vaccinated to unvaccinated birds including wild species. This risk was adequately addressed through the advice on the application of biosecurity measure in the SPC.

Evaluation of the benefit-risk balance

The CVMP concludes that the product is able to reduce air sac lesions and reduce the number of eggs with abnormal shell formation caused by *Mycoplasma synoviae*.

While other clinically relevant objectives for a *Mycoplasma synoviae* vaccine in breeders/layers were not achieved, no major risk has been identified.

The CVMP decided to include information which clarifies that no reduction of respiratory clinical signs, systemic colonisation, and vertical transmission has been demonstrated.

Overall the CVMP therefore considers the benefit risk balance for this product to be favourable.

Conclusion on benefit-risk balance

The benefits of the vaccine as stated above have been sufficiently substantiated. The risks identified for the target species, the user and the environment are considered acceptable. Therefore, the overall benefit-risk balance is considered as favourable.

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considers that the application for MS-H vaccine is approvable.

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.