

SCIENTIFIC DISCUSSION

Product name:	Eurican Herpes 205
Procedure No.:	EMEA/V/C/059/01/0
Applicant company :	Merial
Active substances:	Purified sub-unit vaccine containing glycoproteins of canine herpes virus (CHV).
Proposed International Non-proprietary Name:	N/a.
Pharmaceutical form:	Powder and solvent for emulsion for injection
Strength:	Canine herpesvirus (F205 strain) antigens 0.3 µg to 1.75 µg* *expressed in µg of gB glycoproteins
Presentation/s:	1-dose glass bottle of powder 1 ml glass bottle containing solvent
Package size:	Box of 2 x 10 bottles and box of 2 x 50 bottles.
Target species:	Dogs
Withdrawal period:	Not applicable
Route of administration:	Subcutaneous use
Product type:	Immunological
Therapeutic indication:	Active immunisation of bitches to prevent mortality, clinical signs and lesions in puppies resulting from canine herpes virus infections acquired in the first few days of life.

Introduction

Eurican Herpes 205 is a purified sub-unit vaccine containing glycoproteins of canine herpes virus (CHV). The active ingredient is presented as a powder and supplied with a vial of solvent consisting of an oily adjuvant, for emulsion for injection.

The vaccine is recommended for use in pregnant bitches to provide passive protection of their puppies from the effects of neonatal infection with CHV. The vaccine is to be administered subcutaneously to pregnant bitches at approximately 10 days and 52 days after mating. The 2 dose vaccination regimen is to be repeated in each pregnancy.

Part II A QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

1. Composition of the veterinary medicinal product

1.1 Freeze-dried powder

	Names of ingredients	Quantity per dose
Active ingredients	Canine herpesvirus glycoprotein	0.3 – 1.75 µg gB
Constituents of the excipient	Freeze-drying substrate Buffered physiological saline	
Gentamycin		Trace
Thiomersal		Trace

1.2 Diluent (adjuvant)

	Names of ingredients	Quantity per dose
Constituents of the adjuvant	Oily Phase light paraffin oil	0.33 ml 224.8 to 244.1 mg

II A 2. CONTAINER

The vials used for both the liquid and freeze-dried powder are colourless Type I glass vials, in compliance with the European Pharmacopoeia requirements (3rd Edition 3.2.1). The vials for the freeze-dried powder and the solvent are closed with a butyl elastomer stopper. The stopper is in compliance with the European Pharmacopoeia requirements (3rd Edition 3.2.9). Both vials are sealed with an aluminium cap with a hole in it. Vials are sterilised by dry heat. Stoppers are washed and silicone coated and sterilised by steam.

II A 3. DEVELOPMENT

The Applicant has gained experience over the years from production of glycoprotein subunit herpes viruses for use in other species and is applying these techniques to production of Eurican Herpes 205. The freeze-drying substrate is that used in the other freeze-dried canine vaccines produced by the Applicant and the adjuvant is used in other herpes virus vaccines.

The vaccine strain used for production is one that was isolated in the US from a litter of pups that died from CHV infection. The vaccine is prepared as a purified sub-unit vaccine to ensure its safety in pregnant bitches. The glycoproteins that are contained in the vaccine are considered to be the main immunogenic proteins of the virus, especially the gB glycoprotein, and capable of inducing neutralising antibodies. References are provided in support of this view. Related to this, the Applicant justifies the use of an ELISA test to quantify the gB glycoprotein as the batch potency test and presents the rationale for the protein content used.

The production process is designed to eliminate small molecules, non-specific proteins and proteins not involved in protection. The active substance is a CHV antigen fraction enriched with the viral envelope glycoproteins.

This product is recommended for use immediately after reconstitution and no studies of in-use shelf-life were therefore required.

II B DESCRIPTION OF METHOD OF PREPARATION OF THE FINISHED PRODUCT

II.B.1 FORMULATION

The method of preparation of the active ingredient was presented. The flow chart for production of the purified glycoprotein, that for production of the freeze-dried powder and that for production of the solvent were presented.

II.B.2 DESCRIPTION OF THE STAGES OF MANUFACTURE

Preparation of the active substance

The CHV is grown on MDCK cells. After clarification, it is inactivated. The inactivated virus is then concentrated, purified and dissociated. The capsids are removed and the fraction containing glycoproteins constitutes the active ingredient.

II.B.3 TABLE OF BLENDING DETAILS

1. Freeze-dried powder containing the active ingredient

The flow chart for preparation of the finished vials from the glycoprotein suspension, a table of blending and a description of the process were presented.

The quantity of bulk required to give 1.0 µg gB per 1 ml dose is calculated. The antigen is added to the preparation vessel and held, with stirring at $5 \pm 3^{\circ}\text{C}$.

An appropriate volume of freeze-drying substrate is mixed with sufficient saline to make up the volume of the final bulk.

2. Solvent (adjuvant)

The aqueous phase consists of saline solution and this is added to the preparation vessel to give 0.67 ml per 1 ml volume of bulk solvent. The oily phase is prepared to give 0.33 ml per 1 ml volume of bulk solvent. It consists of light paraffin oil and appropriate excipient.

II.B.4 VALIDATION STUDY RESULTS

1. Inactivation kinetics

Satisfactory studies and information were provided.

2. Sensitivity of the detection system

Satisfactory studies were provided.

3. Consistency of production

a) Active ingredient

Certificates were presented which show the results from the control tests applied to three batches of CHV glycoprotein. These batches were shown to be within the specification proposed.

b) Components of the finished product

Information was provided on the blending of three batches of freeze-dried component from the three batches of active ingredient and also from blending three batches of solvent. The information indicated that the blending was consistent with the method of blending described.

c) Validation data for the purification steps applied and for the dissociation and centrifugation steps
Satisfactory information was submitted. It is considered that the results presented demonstrate this reproducibility.

II C CONTROL OF STARTING MATERIALS

II.C.1 STARTING MATERIALS LISTED IN A PHARMACOPOEIA

A list of starting materials complying with a pharmacopoeia was given, together with information on their use(s) in production and a reference to the page in the dossier where this use is mentioned. For each substance, a copy of the relevant monograph with which the substance is said to comply and a certificate of analysis for a batch, were provided.

II.C.2 NOT LISTED IN A PHARMACOPOEIA

II.C.2.a Biological Origin

A. Seed materials

A number of techniques have been used and different approaches have been taken at different times to test the seed materials for extraneous agents.

1. Madin-Darby Kidney cell line.

Master cell seed

The canine kidney cell line (MDCK) comes from a culture of canine kidney cells established in 1958. Tests conducted include: a) General microscopy, b) Bacterial and fungal sterility, c) Mycoplasma sterility, d) Extraneous viruses, e) Identification of the species, f) Karyology and g) Tumorigenicity and Oncogenicity.

A commitment with regard to sterility testing was made.

CELLS AT THE HIGHEST PASSAGE FOR PRODUCTION

Information was included, where relevant, with the results of the MCS testing. The certificate of results for the testing of the MCS + 20 was presented. Confirmation was provided that the cells of the MCS + 20 passages and the batch of the WCS had the same microscopical appearance as the MCS.

Working Cell Seed (WCSs)

The WCSs are tested for a range of characteristics and extraneous agents in compliance with the EU Table of extraneous agents and the European Pharmacopoeia monograph 0062, Vaccines for Veterinary Use.

The certificate of results for testing a batch of WCS was presented.

A commitment with regard to sterility testing was made.

2. Canine Herpes Virus

Master Seed Virus

Details of the Master Seed Virus (MSV) were provided. The initial strain of canine herpesvirus type 1 was isolated from a litter of pups which died from CHV infection.

Working Seed Virus

Each batch of WSV is tested for a range of characteristics and extraneous agents.

Conclusions

The tests on the MSV and the batch of the WSV have been conducted in a satisfactory manner and meet all the requirements specified in Council Directive 81/852/EEC and relevant guidelines.

B. Substances of animal origin produced on a batch basis

All the substances of animal origin are produced on a batch basis. They are all subjected to treatment and testing.

It can be concluded from the submitted information that the Applicant's proposals for testing substances and treating can be accepted. Concerning BSE, it is accepted that sufficient information and reassurances have been obtained on the sources. Compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been demonstrated.

The microcarriers used in production are made from a thin layer of denatured porcine collagen coupled to a matrix of cross-linked dextran. The pig source is from skins of pigs bred in any European country (not just EU). The microcarriers are sterilised.

II.C.2.b Non-biological Origin

A list of starting materials of non-biological origin and not in a pharmacopoeia was given, together with information on their use in production and a reference to the page in the dossier where this use is mentioned. In all cases, certificates of analysis were provided.

II.C.2.c Media

A table with the two tissue culture media was given. The media are sterilised by filtration through a membrane of nominal porosity $\leq 0.22 \mu\text{m}$.

II D CONTROL TESTS CARRIED OUT AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

1. MDCK production cell cultures

The test for sterility is conducted in accordance with the current requirements of the European Pharmacopoeia; the appearance of the cells is also checked.

2. CHV glycoprotein

A flow chart showing the controls conducted was presented. The inactivated viral harvest is checked for inactivation.

An assay is carried out for the gB glycoprotein content of the finished glycoprotein solution.

In-process controls

These include controls on the filters, recording the time and temperatures of operations, monitoring the sterilisation cycles, checking fill volumes, freeze-drying cycles, appearance of the product after capping, labelling and packing.

II E CONTROL TESTS OF THE FINISHED PRODUCT

Information was presented on the finished product tests. The tests for each component, the techniques involved, a brief description of each test and the limits of acceptance were listed.

Freeze-dried powder of gB glycoprotein

This is controlled by checks and tests on Appearance, pH, Antigen content, Safety, Bacterial and fungal sterility, inactivation and residual humidity.

Solvent

This is controlled by checks and tests on Appearance, pH, Volume, Density, Viscosity, Safety and Bacterial and Fungal sterility. The controls applied can be considered satisfactory.

Batch to batch consistency

A summary of the results from the tests on 3 batches of each component, manufactured in accordance with the method described, was presented. The format of the batch protocols is in accordance with the requirements set out in the EU guidelines on the EC administrative batch release for the implementation of Article 3.3 of Council Directive 90/677/EEC (III/5372/93) and was considered acceptable.

II F STABILITY

II.F.1 Stability of the Finished Product

Freeze-dried powder

Results were presented for 5 batches of the freeze-dried powder, with consecutive batch numbers, tested over 15 months of storage at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. The results presented were for tests conducted every 3 - 6 months for physico-chemical characteristics (appearance, pH and residual humidity) and antigen content.

Further stability data were provided for the freeze-dried powder consisting of data from potency test results conducted on 5 batches up to 27 months. A shelf life of 24 months was considered justified for the vials of freeze-dried active substance.

Solvent

Results are presented for 3 batches of the solvent, with consecutive batch numbers.

The shelf-life of the product, overall, can therefore be set at 24 months.

II.F.2 Stability of the Reconstituted Product

No decrease in potency is expected shortly after reconstitution and the recommendation is to use the vaccine immediately after reconstitution. The stability of the components has been studied. It has been shown that on reconstitution of older freeze-dried powders the density and viscosity are slightly increased but remain within normal limits for the solvent on its own. The safety and efficacy of the reconstituted product was studied in the clinical studies.

III OVERVIEW OF PART III OF THE DOSSIER: SAFETY

III.A. INTRODUCTION

Potential safety risks from this type of product could be associated with the specific and non-specific proteins and with the adjuvant (all of which may produce inflammatory or hypersensitivity reactions). During the development of the product, the Applicant took these risks into consideration and hence the virus is grown in medium without serum, the virus is inactivated, the antigen is purified to eliminate non-specific proteins and the active ingredient consists of sub-units containing mainly the immunogenic proteins to ensure good efficacy and better safety.

Two initial laboratory studies and one further trial and a field trial have been conducted and these, supplemented by scientific arguments, have been presented to address the requirements of Council Directive 81/852/EEC.

III.B. GENERAL REQUIREMENTS

All safety studies were conducted in pregnant bitches and according to the recommended vaccination regimen except for the test in young dogs. All the batches of vaccine used were manufactured in accordance with the description in the dossier (except where excess antigen was included). The laboratory studies were carried out under GLP conditions. The field trial was conducted in accordance with GCP.

Most of the studies have been conducted in seropositive animals because of the difficulties of obtaining seronegative bitches. However supporting evidence of safety from use in seronegative animals was also provided. No adverse systemic effects or adverse effects on pregnancy were observed in any of the studies. A range of types of local reaction were seen in the different studies, due, in part at least, to the differences in the way they were conducted. The warnings in the SPC on the local reactions that might be expected after administration were agreed, on the basis of all the information available. It was demonstrated that there was no apparent increase in reactions after administration of four repeated administrations of a single dose.

III. C. Laboratory studies

III.C.1 Safety of the Administration of a Single Dose

The objective was to study the effect of the vaccine on bitches and on the development of their foetuses and pups until 8 weeks of age. The vaccine batch used contained low quantity of active ingredient per dose and multiple doses of the freeze-dried powder were used so that a sufficient amount of active ingredient was administered. Twenty bitches were randomly allocated to 3 Groups. All the bitches had antibodies before vaccination. All were primiparous. A date of mating (D_0) was calculated for each group of bitches from the dates of three successive matings. Ten days after this presumed date of mating (D_{10}), the 8 bitches in Group 1 and 6 in Group 2 were given large of amount gB from 4 vials of freeze-dried powder, reconstituted with 2 doses of solvent (i.e. just over a maximum dose of antigen and a double dose of adjuvant).

Six weeks later, (D_{52}), the 7 bitches from Group 1 and 4 from Group 2 that had been shown to be pregnant, received the contents of 2 vials of freeze-dried powder reconstituted with 1 vial of solvent. Bitches in Group 2 also received another vaccine at a different site on D_{10} . All injections were made through an area of skin that had been clipped on the previous day. The 8 bitches in Group 3 received no vaccinations. General clinical examinations and detailed examinations including rectal temperatures and the detection of injection site reactions were made on the day before injections, immediately before injections, 4-6 hours later and daily for 14 days after each injection. Assessment of local reactions included grading for pain, swelling, local heat and itching. Body weights were recorded and food consumption noted. Pregnancy diagnosis was performed 5-6 weeks after mating. After parturition, litters were monitored and pups were examined daily and weighed on day 3 and 7 and then weekly until 8 weeks of age. Dead or sacrificed pups were post-mortemed.

Blood samples were collected on D_{10} and D_{52} . All the vaccinates had higher titres by D_{52} . All but one of the controls had lower titres on D_{52} , indicating a lack of exposure to disease during the study. No adverse general clinical signs or hypersensitivity reactions were noted after vaccination of the bitches. Most vaccinates had a slight increase in rectal temperature in the 15 days post-vaccination. The time of maximum increase was variable; there was no significant difference between the groups and some animals had had higher than normal rectal temperatures before vaccination. It was concluded, therefore that the increases in rectal temperature were not vaccine related. It was also concluded that there was no vaccine related differences in the body weights of the bitches in the groups.

There was no adverse effect on pregnancy, whelping, perinatal mortality or development of the pups between the groups. There were no vaccine-related findings on post-mortem examination of dead pups. Local reactions observed in Group 1 after the first vaccination were slight erythema in most bitches which persisted in two animals to beyond the end of the observation period and there was blackish discolouration in one case. The reactions in Group 2 were more pronounced and the

reactions in half the bitches included scabs which persisted in 2 animals beyond the end of the observation period. The bitches in Group 2 had had a distemper/hepatitis/parvo/parainfluenza vaccine with *Leptospira* administered at a separate site on D10 and this may have been the reason for this greater reaction. The reactions to the other vaccine were broadly similar to those from Eurican Herpes 205. The only reaction observed after the injections on D52 was slight erythema in 1 bitch in Group 1.

Post-mortem and histological examination of the injection sites were also undertaken for 6 animals in Group 1 and 3 animals in Group 2 but not until at least 85 days after the first injection. No abnormal lesions were noted.

The Applicant's conclusions on a lack of significant effects on parameters such as weight gain and increases in rectal temperatures post-vaccination are acceptable. The data provide supporting evidence that there were no detectable general adverse effects on the bitches or their offspring from use of the product in immune, pregnant bitches.

III.C.2 Safety of a Single Administration of an Overdose

The objective was to study the safety of a repeat administration of an overdose of the vaccine in pregnant bitches. The vaccinates were studied for signs of clinical adverse reactions and to monitor the effects on their pregnancy, gestation and offspring. The vaccine batch used in this study was above maximum potency.

Twenty-seven beagle bitches, were randomly allocated to 2 groups of 13 and 14 dogs. A date of mating was estimated for each bitch and 10 days later (D0), the 13 bitches in Group 1 were given a double dose of freeze-dried powder, reconstituted with 2 doses of solvent. Bitches in group 2 were given a placebo vaccine containing freeze-dried vaccine stabiliser reconstituted with the adjuvant solvent. Six weeks later, (D42), a second double dose of vaccine was administered to the bitches in Group 1 and stabiliser given to the bitches in Group 2.

On the 3 days before each vaccination, the animals were clinically examined; the examination included the recording of rectal temperatures. Only healthy bitches with rectal temperatures $\leq 39.5^{\circ}$ C were included in the trial. After each injection, the animals were monitored for 14 days to monitor the effects on general condition and for injection site reactions, and rectal temperatures were recorded for 4 days. Parturition occurred between 3 -13 days after the second dose of vaccine in Group 1 and between 1 -19 days after the second dose of placebo in Group 2. Parturition was monitored, the number of live, dead and abnormal pups born was recorded and the pups were followed for 8 weeks. Unexpected deaths were investigated with post-mortem examination.

No increases in rectal temperature were found in any bitch. No general adverse effects were noted except depression on day 48 in 1 control animal. Local reactions were recorded at the injection site from one or other or both vaccinations in about half the bitches in both groups. These were described as slight and transient swellings and all persisted for less than 4 days. They were recorded as oedema ≤ 4 cm and occurred between days 1 and 4 post-vaccination. One dog in Group 2 had irritation at the site on D6 after the first dose. There was no pain on injection, local hyperthermia or loss of pigment or hair at any of the injection sites.

No statistically significant differences were found between the two groups with respect to the total number of pups born, the numbers of live/dead pups, or the number weaned, nor between the reproductive performance of the multiparous animals in Groups 1 and 2 and their historical data. The data provide evidence of the general safety of a double dose of the product when administered to immune, pregnant bitches according to the recommended vaccination schedule. There were no detectable adverse effects on the bitches or their offspring. The local reactions which developed after the vaccinations and administration of the adjuvant indicate that these are acceptable reactions to the adjuvant. In the overdose study, slight and transient swelling was noted. It was considered by the Applicant that the reactions seen in these two studies were, in fact, similar reactions to the injections which contained a double dose of adjuvant in both cases.

It has been confirmed that the general and local reactions which occurred after the vaccinations of the non-pregnant bitches was the same or very similar to those reported for the pregnant bitches.

III.C.3 Safety of the Repeated Administration of a Single Dose

- a) The two studies summarised above are stated as also demonstrating the safety of the repeated administration of a single dose and an overdose. It is accepted that there was no evidence in either of these studies that two administrations of the vaccine to immune animals resulted in a higher rate of local reactions after the second dose or that the vaccinations produced detectable general reactions or signs of a hypersensitivity reaction.
- b) The objective was to study the safety of a repeat administration of a single dose of the vaccine in young herpes-free dogs. The vaccine batch used in this study contained gB above the maximum potency. The test was conducted in seronegative young dogs because of the difficulties in obtaining seronegative bitches. It is considered that the use of these dogs would not impair the detection of local or general adverse reactions or hypersensitivity reactions from multiple injections.

Fifteen beagle puppies were randomly allocated to 2 groups of 11 and 4. Blood samples were collected immediately before the first injection (D0) to check (by ELISA) that they were free from antibodies to CHV. All the puppies had been vaccinated previously with another vaccine. The 11 puppies in Group 1 were vaccinated, subcutaneously, with a single dose on days D0, D14, D28 and D42. The control pups were left unvaccinated. The animals were examined for local reactions, general condition and rectal temperatures recorded daily from D0 until the end of the observation period on D56 as well as 4 hours after each injection. The pups were also weighed at intervals. The classification for general reactions and the scoring system for local reactions were presented.

The results for rectal temperatures were presented. There were no notable differences in the average results of the two groups but there was transient hyperthermia (up to 39.8⁰C) in 6 of the vaccinates and in 2 of these this was seen on a number of occasions. Transient hyperthermia (up to 40.5⁰C) was also seen in 1 of the controls. It was considered that this modest hyperthermia was not related to vaccination.

The results for the average weights in the groups were presented. There were no notable differences in the average results of the two groups but 5 vaccinates showed some weight loss – 1 between D0 and D7, 1 between D14 and D21 and 3 between D42 and D49. There were no other associated side effects in any of these dogs. No general adverse effects were noted in any dog.

The only local reactions recorded were some swellings at the injection sites in the vaccinates. The largest reaction was a very diffuse 5 cm swelling. One dog did not display any reaction. Most reactions seem to have been recorded after the second and third injections. After the fourth injection only 1 dog had a small reaction 4 hours after vaccination.

The conclusion drawn by the Applicant was that a few local reactions were observed, probably related to the oily solvent but they remained small and were not systematically observed.

The combination of the results from this study and the results of the safety studies in pregnant bitches is considered to provide an acceptable alternative to a study of the safety of the repeated administration of the vaccine to pregnant bitches.

III.C.4 Examination of Reproductive Performance

The study reported under III C 1 is also stated as demonstrating the absence of adverse effect of the vaccine on the reproductive performance of the vaccinated bitches. The results from these two trials plus data from two field safety and efficacy studies were assessed and the outcome presented. The comparison was based on the results from 171 vaccinated bitches and 95 placebo treated controls, of different breeds and ages and from 14 kennels with differing CHV status. A check was made on the homogeneity between the groups, in terms of the age and size of the bitches. There was no significant difference but differences were taken into account in the analyses of the results. The reproduction parameters considered were rate of pregnancy, number of pups born (live and dead) and weaned.

The summary data for the size, age and breed of the animals in the trial were presented. The pregnancy rates of vaccinates and controls were not significantly different. The number of fetuses was only correlated to the age and size of the bitches. The overall number of weaned pups was not

significantly different between the two groups. There was no correlation between vaccine potency and any of the parameters studied. The CHV status had an effect.

The analysis confirms the conclusions reached from the two laboratory safety studies that there is no detectable adverse effect on these specific reproduction parameters when the vaccine is administered to immune pregnant bitches.

In addition to the points made above, it was stated that 15 batches had been submitted for batch safety testing in SPF dogs without noticeable adverse effects. More than 50 bitches with no or low antibody titres had been vaccinated in the field trials without any adverse effect on their reproductive performance. It can be concluded that the Applicant has provided sufficient evidence of the safety of the product including safety for use in pregnant bitches even if non-immune.

III.C.5 Examination of Immunological Functions

Satisfactory argumentation has been provided to show that there is no reason to expect the vaccine would exert an adverse effect on the immune function of the vaccinated animals. In support, a lack of adverse effect noted from use of the Applicant's other herpes virus vaccines in other species was quoted.

III.C.6 Special Requirements for Live Vaccines

Not applicable.

III.C.7 Study of Residues

Not applicable.

III.C.8 Interactions

The wording "No information is available on the efficacy from the concurrent use of this vaccine with any other product" was accepted.

III.D. FIELD STUDIES

1. The objective was to evaluate the safety of administration of the recommended vaccination schedule in pregnant bitches. The vaccinates were studied for signs of clinical adverse reactions and to monitor the effects on their pregnancy, gestation and offspring. The vaccine batch used was above the maximum potency. The control animals were given a placebo vaccination containing freeze-dried vaccine stabiliser reconstituted with the adjuvant solvent.

The study was conducted in the resident, CHV seropositive breeding bitches on two commercial breeding kennels. There were 52 beagles on site 2 and 15 animals of a range of pedigrees on site 1. On each site, the animals were randomly allocated to the group of vaccinates or the placebo treated group in a ratio of 2:1.

On site 1 the first injections were given between D4 and D13 after mating and pregnant animals were given a second dose of vaccine or placebo between D47 and D56 after mating. On site 2, the injections were given on D10 and D52.

The animals were closely monitored on the day before injection, immediately before injection, 3-5 hours after each injection and daily for 4 days after each injection, for effects on general condition, rectal temperatures and injection site reactions. The general condition of the bitches was monitored from day 14 to 52 and day 57 to whelping. Parturition was monitored, the number of live, dead and abnormal pups born was recorded and the pups were observed for 3-6 weeks.

Vaccination had no effect on the general condition of the bitches. There was no statistically significant difference on the effect on rectal temperatures between the groups. There were no adverse

effects at the injection sites, except three of the vaccinated bitches on site 2 developed transient swellings after the first injection. These first appeared on day 0, 1 or 2 post vaccination. They were scored as ≤ 4 cm and recorded as present on 4 or 5 consecutive days, after which they appear to have resolved.

There were no statistically significant differences in pregnancy rates, duration of gestation, the numbers of pups born or the number weaned. The statistical analysis of results was presented.

This field trial was conducted in conformity with Specific requirements for the production and control of live and inactivated vaccines for cats and dogs (III/5736/94) and the requirements of Council Directive 81/852/EEC. The trial can be considered as having provided sufficient evidence of a lack of detectable adverse effect of the vaccine when used under field conditions, in premises where the bitches were infected with or had been exposed to CHV infection.

2. The Applicant also refers to the data for the 144 bitches vaccinated under field conditions as showing the very good safety associated with use of the recommended vaccination schedule.

III.E. ECOTOXICITY

A first phase environmental risk assessment was presented. The product does not present a risk for the environment and no second phase study is required.

IV. OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY

IV.A INTRODUCTION

Two laboratory studies and two field trials were carried out. In one field trial the serological response was measured. In the other, a study was made of the effect of vaccination on the reproductive performance of bitches in an infected kennel. These trials, supplemented by scientific arguments, have been presented to address the testing requirements of Council Directive 81/852/EEC.

IV.B. GENERAL REQUIREMENTS

All efficacy studies were conducted in pregnant bitches (except for the dose response study) and according to the recommended vaccination regimen. All the batches of vaccine used were manufactured in accordance with the description in the dossier (except where less than the usual amount of antigen was included). All studies were carried out under GLP or GCP conditions.

IV.C. LABORATORY TRIALS

1. Dose response study

The objective was to study the dose-response effect of the vaccine in dogs, using serology as the means of measurement. The vaccine batch used contained gB at minimum potency.

Fourteen SPF dogs, aged 6 months were allocated to 3 groups of 4 dogs and one group of 2. On D0, dogs in Group 1 were each given 2 doses of active substance and 1 ml of solvent, Group 2 dogs were given 1 dose of active substance and 1 ml of solvent, Group 3 dogs were given half a dose of active substance and 1 ml of solvent and the two dogs in Group 4 were each given 1 ml solvent, only. The injections were repeated on day 42. The vaccine doses were prepared in pools with the necessary quantities of adjuvant to give the necessary ratio of antigen per 1 ml of solvent. Seroneutralising antibody titres at D0 were measured in \log_{10} and ranged from 0.2 to 0.6 (mean 0.29). By 2 weeks after the second vaccination, the means in the vaccinates had risen and the controls remained low. The mean responses in the vaccinates had decreased slightly by D98. A linear relationship between

antigen dose and serological response was established from the results obtained. The conclusion was that a dose between 1 and 1.5 of the batch tested should provide a satisfactory choice for a minimum protective dose.

Reference was made to the titre of 0.6 log₁₀, which was established in a study published by Huxsoll on the titre required in bitches to protect their pups. A safety margin has been added. From the linear relationship of antigen and serology established, the Applicant had defined the minimum potency which has been tested further.

This study provides information on the antigenicity of the CHV glycoprotein. The dose-response effect is detectable and appears to provide a reasonable basis for choosing the minimum antigen level gB for the vaccination and challenge study.

The study was continued to observe antibody titres until day 379. A table of results was presented, with the mean antibody responses in the three groups of vaccinated dogs and the controls from samples collected at day 407 and day 421 (together with the results from the earlier times of sampling, previously presented). A single booster dose had been given on day 407 and this had produced no more than a moderate response in all groups, 14 days later.

The results support the recommendation that two vaccinations during each pregnancy is preferable to ensure high titres at the time of whelping.

2. Vaccination-challenge study

The objective was to study the efficacy of the vaccine by immunising pregnant bitches to protect passively their puppies against a CHV challenge. The vaccine batch used contained minimum quantity of gB per dose.

Sixteen pregnant SPF bitches, were used. Approximately 10 days after the presumed date of mating, 10 bitches were vaccinated, 5 were given solvent only and 1 bitch received no injections. A second injection of the vaccine or solvent was administered 52 days later to those animals that were still included in the trial.

The litters of 6 vaccinates and 6 controls were challenged at 3 days of age. (The litters of four of the vaccinates were not challenged for various reasons, not connected with the vaccine.) The challenge was a virulent strain of CHV, given oronasally. Most pups were observed daily for clinical signs of disease until 21 days after challenge (D24). One litter of a control bitch was only observed for 2 weeks. Post mortem examination was carried out on pups that died before D24 and on the surviving pups, sacrificed on D24. Histological examination was conducted on samples collected at post-mortem on D24 and on tissues from some other pups that died earlier including the organs of 1 control litter with classical CHV lesions at post-mortem which was then used as a positive control for histology. Samples of lung and kidney were pooled and tested for viral isolation and for the presence of virus by PCR (polymerase chain reaction). In some cases, additional tissue samples were added to the pool.

The clinical signs, which were particularly looked for after challenge, were signs of respiratory disease (sneezing, discharge, and respiratory distress), diarrhoea and vomiting, general signs of distress and illness in young pups and morbidity. Blood samples were collected from the bitches at the time of each vaccination, at the time of challenge and at the end of the study on D24. Surviving puppies were tested on D24 post-challenge. The serum-neutralisation test was used to assess antibody titres.

Pups were diagnosed as succumbing to neonatal CHV infection on the basis of death associated with typical macroscopic lesions at post-mortem examination. Isolation of virus and/or positive PCR was used for confirmation.

Post-mortem findings which were considered to be typical macroscopic lesions of neonatal CHV disease were described. The general appearance of the pup was often normal, sometimes thin; abnormalities including petechiae are seen in the liver, spleen, kidneys, digestive tract, mesenteric ganglion and heart, and the lungs have a heterogeneous appearance (reddish areas, greyish areas, oedema and 'watery').

For the controls the bitches were seronegative and all but one seroconverted after challenging the pups. 18/29 pups died after challenge (excluding 2 deaths considered non-specific). In 5/6 litters part or all of the litter succumbed to the infection. Many died quickly without characteristic clinical signs

being observed. Pups dying between days 6 and 14 had typical macroscopic post-mortem lesions. Virus was isolated from samples from nearly all of these. In the sixth litter, 1 pup died 1 day post challenge and this was considered a non-specific death. The other three pups in this litter survived and although there were signs of illness and the samples collected on D24 were PCR positive they were included in the group considered protected. The antibody levels in surviving pups were highly variable.

With regard to the vaccinates, the bitches seroconverted to the first vaccination, showed a booster effect to the second and all had high antibody titres by the time of challenging the pups. The date of challenging the pups was not always 56 days after the first injection as the bitches whelped from 7 to 16 days after the last injection. After the challenge of the pups, the antibody titres of the bitches stayed the same in 4 and increased in 2. No pups were diagnosed as having died of CHV infection. The 6/33 that died were considered to have died from other causes. The antibody levels in surviving pups were highly variable.

The indication "Active immunisation of bitches to prevent mortality, clinical signs and lesions in puppies resulting from canine herpes virus infections acquired in the first few days of life" was agreed.

3. Duration of immunity

The vaccination regimen is designed to ensure a satisfactory neutralising antibody titre in the bitch at the time of whelping and for a few days subsequently since pups gain protection by absorbing IgG from the colostrum and milk for the first 12 - 36 hours of life.

Since the recommendation is for the vaccination regimen to be repeated each pregnancy the duration of immunity beyond whelping and the efficacy of a single booster dose has not been addressed by the Applicant. Justification has been provided for the repeat of the two dose vaccination. It was shown that there was an inadequate response in bitches given a single booster dose.

IV.D. FIELD TRIALS

Two field trials were conducted and the results are summarised below.

1. The objective was to study the efficacy of administration of the recommended vaccination schedule in pregnant bitches. Efficacy was assessed through monitoring the serological response to vaccination. In this study, ELISA antibody titres were measured whereas seroneutralisation titres were measured in the laboratory efficacy studies. A report was provided with the details of the comparison of the two methods (see below) Confirmation was provided that ELISA titres were used throughout this study. The vaccine batch used contained approximately twice the minimum potency. The control animals were given a placebo vaccination containing freeze-dried vaccine stabiliser reconstituted with the adjuvant solvent.

The study was conducted in 152 bitches in ten breeding kennels having variable status for CHV infections. There were approximately 18 different breeds in the trial. On each site, the animals were randomly allocated to the group of vaccinates or the placebo treated group in a ratio of 2:1. The 100 bitches allocated to Group A and the 52 placebo treated bitches in Group B were injected on approximately D10 and D52 post-mating. All bitches were checked and shown to be in good general health before each vaccination. Non-pregnant bitches and one too close to whelping by D52 were removed from the study and no data were presented for these 25 vaccinates and 17 controls. Blood samples were collected before each vaccination and within 7 days after whelping. The statistical analyses applied to the results of antibody titres were listed.

The individual titres for each dog were given by kennel then by group. The details of the statistical analyses were also provided. Antibodies were present at variable levels at the time of first vaccination in all the bitches from both groups and there were no statistically significant differences between them. In some kennels most or all of the bitches had low antibody levels before injection. In others there was a mixture of bitches with low and high levels.

Controls: The controls had average titres at the three times of sampling with no significant differences between the titres at the various times of sampling. The titres of most bitches stayed the same or decreased and only 1 bitch in kennel B clearly seroconverted from D10 to after whelping.

Vaccinates: A significant increase in the average antibody titre had occurred after the first and second vaccinations and the average antibody titre was significantly higher after whelping than on D10. There were three non-responders (in one case the titre had markedly decreased) and five bitches with a poor response but the majority of the bitches had increased titres after vaccination. There does not appear to be any relationship between titres at D10 and those induced by the vaccination in that bitches with low and higher titres at D10 developed high titres after whelping.

It was concluded that the vaccine induced a strong seroconversion. The lack of response in the controls indicates that there was not an outbreak of CHV in the kennels that could have boosted the antibody titres in the vaccinates.

The efficacy of the vaccine has been demonstrated in a vaccination/challenge study with a vaccine at minimum potency, administered according to the recommended vaccination schedule. The claims being made relate to protection against the disease and not to specific induction of antibody titres. It was concluded that sufficient efficacy data had been provided in support of the claims being made.

Validation of the serological test

Ten SPF dogs, aged approximately 7 months were allocated to 2 groups of 5. On D0, dogs in group A were each given 1 dose of a vaccine batch and Group B dogs were given 1 dose of another batch. Both were reconstituted in 1 ml of solvent. The injections were repeated on D42. Blood samples were collected before vaccination on D0 and on D28, D42, D56 and D70.

Serology

Mean titres rose from D0 to D28, decreased slightly by D 42 then increased markedly to D56 and decreased slightly again by D70. Mean ELISA titres were higher than seroneutralisation titres. On the whole, the results for the individual dogs follows the pattern of the means and there were no non-responders.

A linear relationship was demonstrated between the titres obtained from the two assay methods. This provides sufficient reassurance that the ELISA titres measured in this field trial can be used as an indicator of the immunogenicity of the vaccine and its efficacy.

2. The objective was to study the efficacy of administration of the recommended vaccination schedule in pregnant bitches. Efficacy was assessed through monitoring the reproductive performance in bitches in an infected kennel with reproduction problems. The vaccine batch used was at approximately twice the minimum potency. The control animals were given a placebo vaccination containing freeze-dried vaccine stabiliser reconstituted with the adjuvant solvent.

The study was conducted in 20 bitches in the breeding kennel. CHV infection had been confirmed on the site by virus isolation and serological assessment. The animals were randomly allocated to the group of vaccinates or the placebo treated group in a ratio of approximately 2:1. The 14 bitches in Group A were vaccinated on approximately D10 and D52 post-mating. The actual planned vaccination dates were between D7 – D15 but 1 animal was injected on D20. For the second injection it was planned for D49 - D55 but 3 animals were injected slightly outside this timeframe. The 6 placebo treated bitches in Group B were injected to the same time schedule. Non-pregnant bitches were removed from the study and no data were presented for these 4 vaccinates and 2 placebo treated bitches except for some serological titres.

The animals were examined and rectal temperatures recorded before each injection. Blood samples were collected before each vaccination and a few days after whelping. Antibodies were measured by ELISA.

The vaccinates were monitored for their rate of pregnancy, problems at whelping, the incidence of stillborn pups, general condition and mean body weight of the pups at whelping, pre-weaning losses, general condition over 6 weeks and mean body weight of the pups at 6 weeks of age. Although it is known that all these reproduction parameters can be affected by CHV infection in the kennels, only pre-weaning losses were relevant to the claims being made for the product. Post-mortem examinations were conducted on puppies that died and virus isolation was conducted from various organs.

There were no significant differences in the rate of pregnancy or duration of gestation. There were no abortions or whelpings before D58 and there was no difference observed in whelping difficulties. There were no significant differences in the number of fetuses per bitch. Four stillborn pups were seen in the control group and 2 in the vaccinates. All but one of the pups which were less than 100g at birth were stillborn or died within 48 hours. Although it appears from the data presented that the rate of survival over the first 48 hours was better in pups from vaccinates compared to the controls, the difference was not statistically significant. No CHV isolations were obtained from any dead pup. The mean body weight at whelping between pups born to vaccinates was highly significantly greater than those born to the controls (mean 141.8 g compared with 85.0 g) but by weaning the differences were not significant. The surviving pups in Group B grew faster.

The mean ELISA antibody titres were similar in the two groups at the time of first vaccination. No control bitch seroconverted from D10 to after whelping and most titres stayed the same. There were

1 or possibly 2 non-responders but the majority of the vaccinated bitches had higher titres after vaccination and after whelping. These results show that the vaccine at an antigen content approximately double the minimum, is able to stimulate an immune response and raise the circulating antibody levels even in bitches which had a reasonable titre before vaccination.

It was concluded that the efficacy of the vaccine has been confirmed in field conditions, based on serological results, and the trials also showed some evidence of protection against the CHV induced effects on pregnancy (e.g. from the increased mean body weights of the pups at birth).

5. RISK-BENEFIT ASSESSMENT AND CONCLUSION

Eurican Herpes 205 is a vaccine containing purified glycoproteins of canine herpes virus (CHV). The product consists of 2 vials. The freeze-dried active substance is supplied in 1 vial. The solvent, which includes an oily adjuvant (light paraffin oil) is contained in the second. The active substance is freeze-dried in vials. The vials used for both components are made from Type I glass. Both the freeze-dried pellet of the active substance and the solvent have been shown to be stable over a period of storage of 27 months at 2-8 °C. This justifies a shelf-life of 24 months for the components and the product. The information supplied has provided assurance that the control tests will control the quality and quantity of the glycoprotein in the freeze-dried powder including any variation in the quantity of glycoproteins other than the gB content. All materials of biological origin are sourced in accordance with the Note for Guidance for Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Veterinary Medicinal Products, EMEA/CVMP/145/97-Revision.

The vaccine is indicated for use in pregnant bitches to provide passive immunity to their pups. The vaccination schedule stated in the SPC is for vaccination 7-10 days after the presumed date of mating with a second dose 6 weeks later. All but one of the safety studies were conducted in pregnant bitches and according to the recommended vaccination regimen. The laboratory studies were carried out under GLP conditions. The field trial was conducted in accordance with GCP. Three laboratory studies, a field trial and a statistical analysis have been conducted to establish safety. Most of the studies have been conducted in seropositive animals because of the difficulties of obtaining seronegative bitches. Supporting evidence of safety from use in seronegative animals was also provided. No adverse systemic effects or adverse effects on pregnancy were observed in any of the studies. A range of types of local reaction were seen in the different studies, due, in part at least, to the differences in the way they were conducted. Reactions at the site of injection have been observed but are not considered so significant as to cause a major concern regarding the welfare of treated animals but warnings regarding the local reactions that might be expected after administration have been included in the SPC. It was demonstrated that there was no apparent increase in reactions after administration of four repeated administrations of a single dose.

Operator warnings for the oil adjuvant have been included in the SPC and some of the product literature. The overall risk to the environment is assessed as minimal.

Two laboratory studies and two field trials were carried out to establish efficacy. In one field trial the serological response was measured. In the other, a study was made of the effect of vaccination on the reproductive performance of bitches in an infected kennel. The first laboratory study was a dose response study to establish the antigen content likely to be required in a dose to provide protection. The second study was conducted to demonstrate the protection of puppies from vaccinated dams from a CHV challenge. Good protection was achieved. Over 80% of the pups from the vaccinated bitches survived and those pups that died were not considered to have died as a result of CHV infection. On the other hand, 66% of the controls died.

The Applicant recommended a repeat 2 dose vaccination in each pregnancy. This approach was accepted as the interval between pregnancies is variable and can be longer in some breeding kennels than in others. In addition, it was shown that the immunity of the bitches wanes to a very low level at or before 12 months post vaccination and a single booster dose was not then effective. The data on the duration of immunity in pups is limited to 3 days post whelping, as this was when the challenge was administered in the study. This was, however, considered acceptable as the pups become increasingly resistant to the effects of infection over the first 2-3 weeks of life. The claim being made reflects the data generated and reads "Active immunisation of bitches to prevent mortality, clinical signs and lesions in puppies resulting from canine herpes virus infections acquired in the first few days of life". Based on all the data provided, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were in accordance with the requirements of Council Directive 81/852/EEC and the data supported the claims made.
