SCIENTIFIC DISCUSSION

Name of the medicinal product:	Vaxxitek HVT + IBD
Marketing Authorisation Holder:	MERIAL 17, rue Bourgelat, 69002 LYON, FRANCE
Active substance:	Live vHVT013-69 recombinant virus, at least 3.0 $\log_{10}$ PFU
International Nonproprietary Name:	Not applicable
Pharmaco-therapeutic group (ATC Code):	Immunological QI01AD15
Therapeutic indication(s):	For active immunisation of chickens: • To prevent mortality and to reduce clinical signs and lesions of Infectious Bursal disease. The onset of protection is from 2 weeks and the protection extends to 9 weeks. • To reduce mortality, clinical signs and lesions of Marek's disease. The onset of protection is from 4 days. A single vaccination is sufficient to provide protection during the risk period.
Target species	Day-old chickens and 18 day embryonated eggs.

III. SCIENTIFIC DISCUSSION

INTRODUCTION

Vaxxitek HVT + IBD is a live vaccine based on the use of a recombinant turkey Herpesvirus (HVT) expressing the VP2 gene of the Infectious Bursal Disease (Gumboro disease) Virus (IBDV). The product contains an organism within the meaning of Article 2 (1) of Council Directive 90/220/EEC. Written consent from the competent authorities from France to the deliberate release into the environment of the GMO for the purpose of research and development was provided. The pharmaceutical form is a frozen suspension with solvent for injection.

Each dose of vaccine (0.2 ml) contains at least 3.0 log10 PFU of the live vHVT013-69 recombinant virus. It must be administered by means of a single subcutaneous (S.C.) injection.

The indication is for the active immunisation of day-old chickens as follows:

- To prevent mortality, to reduce clinical signs and lesions of Infectious Bursal disease.
  The onset of protection is from 2 weeks and the protection extends to 9 weeks.

- To reduce mortality, clinical signs and lesions of Marek's disease.
  The onset of protection is from 4 days. A single vaccination is sufficient to provide protection during the risk period.

Marek's disease is the most common lymphoproliferative disease of chickens. HVT strains are commonly used to vaccinate day-old birds. The parental strain of Vaxxitek HVT + IBD (FC-126 strain) is widely and classically used in vaccination against Marek's disease. Infectious bursal disease is widely spread world-wide, and particularly where the avian industry is intensive. It induces a severe immunosuppression (marked tropism for the bursa of Fabricius), and the infection is followed by diarrhoea, illness, dehydration, and muscular haemorrhages. Morbidity is very high (50 to 100%) in young animals and mortality depends on the virulence of the strain (0 to 50%) and the presence of maternally derived antibodies (MDA).

Vaccination with classical Gumboro live vaccines must be carried out in very young animals, with two or more administrations. To ensure a sufficient protection against very virulent (vv)IBDV strains, "hot" vaccinal strains are more and more used, which result in safety and immunosuppression problems. As opposed to attenuated live IBD vaccines, highly susceptible to MDA, Vaxxitek HVT + IBD does not show any interference by MDA. It can be thus administered to day-old chickens, without presenting any safety problem or inducing immunosuppressive effect. All biological and immunological properties of the parental strain are retained.

The vaccine is a frozen suspension presented in sealed ampoules, to be reconstituted in a diluent. Ampoules are clipped on metallic carriers. Carriers are stored and sold in liquid nitrogen containers. Diluent is stored at room temperature. The containers belong to Merial, and empty containers may come back to Merial when new ones are delivered to hatcheries or when reconditioning is required. Some large scale hatcheries have their own liquid nitrogen tank and carriers are directly transferred when delivered.

The conditions of production have direct consequences on labels, since the primary packaging of the vaccine suspension is very small (2 ml-ampoule), and there is no possibility to perform any labelling operation after freezing. The diluent is presented separately and the labelling operation for polyvinylchloride bags is performed before filling and sterilisation (it cannot be performed afterwards due to the protective overpouch film).

Merial will ensure the connection between the container contents and the package inserts by implementation of GMP at all steps including the packaging step. Furthermore, the link between

container contents and package inserts may be simply checked by the well established colour coding system for ampoule carriers.

The Marketing Authorisation was extended in March 2004 to include the *in ovo* route of administration.

OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

II.A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE COMPONENTS

Vaxxitek HVT + IBD vaccine is a live recombinant vaccine against Gumboro disease (or infectious bursal disease) and Marek's disease in chickens. It is a frozen vaccine to be diluted with an aqueous diluent. The active ingredient is the production-cell associated vHVT013-69 virus vaccine strain, a recombinant turkey Herpesvirus (HVT) expressing the VP2 coding sequence of Infectious Bursal Disease Virus (IBDV). The parental strain of Vaxxitek HVT + IBD (named FC-126) belongs to the serotype 3 of Marek's disease and is widely used in classical vaccination against Marek's disease. HVT strains are apathogenic in all species, and do not replicate in mammalian cells. The inserted sequence corresponds to the sequence coding for the VP2 protein of Infectious Bursal Disease Virus (IBDV). It has been cloned from 52/70 Faragher strain. The VP2 protein is the only protein of IBDV that induces protection against Gumboro disease.

1. Composition

1.1. Frozen viral suspension

Active substance : vHVT013-69 component 3.0 0 $\log_{10}$ PFU[1] per dose.

Constituents of the excipient	Dimethyl sulfoxide	Ph.Eur.
	Dilution medium	MERIAL
	composition for 1 ml: F10 HAM medium	MERIAL
	199 medium	MERIAL
	TPB	MERIAL
	Foetal calf serum	MERIAL
	Calf serum	MERIAL
	Sodium hydrogen carbonate	Ph. Eur.
	Phenol red	MERIAL
	Hydrochloric acid	MERIAL
	Purified water	Ph. Eur.

1.2. Diluent

Sucrose

Casein hydrolysate
Dipotassium phosphate
Potassium dihydrogen phosphate
Phenol red sodium salt
Sodium hydroxide
Water for injection

2. Containers

The ampoule for frozen suspension is compliant with the European Pharmacopoeia (Type I glass, flame sealed). The bottle for the diluent is polypropylene (200 ml and 500 ml volumes) and is Ph.Eur. compliant. The closure for bottle diluent is made from nitrile elastomer (Ph. Eur.) and sealed with an

[1] PFU = Plaque Forming Units

operculated aluminium cap. The bag for diluent is polyvinylchloride (250 ml, 500ml, 1,000 ml, 2,000 ml and 3,000ml)(Ph. Eur.). Sealed after filling, each bag is placed in a polyethylene protective overpouch heat-sealed with two connecting tubes. The content is accessible through a septum.

3. Development Pharmaceutics

3.1. Choice of the strain

The active ingredient is the production-cell associated vHVT013-69 virus vaccine strain, a recombinant turkey Herpesvirus (HVT) expressing the VP2 coding sequence of Infectious Bursal Disease Virus (IBDV). The parental strain of Vaxxitek HVT + IBD (named FC-126) belongs to the serotype 3 of Marek's disease and is widely used in classical vaccination against Marek's disease.

HVT strains are apathogenic in all species, and do not replicate in mammalian cells. The inserted sequence corresponds to the sequence coding for the VP2 protein of Infectious Bursal Disease Virus (IBDV). It has been cloned from 52/70 Faragher strain. The VP2 protein is the only protein of IBDV that induces protection against Gumboro disease.
The insertion of the gene coding for the Gumboro VP2 protein in the HVT parental strain allows the preparation of a vaccine that can be administered to day-old chickens without presenting any safety problems or immunosuppressive effect.

The vaccine is administered during a short period of time (at most 2 hours following reconstitution as indicated in the SPC) using high speed automated or semi-automated systems (up to 2000 birds vaccinated per hour). This short period of time is unlikely to support contamination. The lack of a preservative in the multi-dose containers was, therefore, justified.

In order to recommend a new route of injection (*in ovo* route), the Applicant increased the minimum titre from 3.0 log10 PFU/dose to 3.6 log10PFU/dose, based on efficacy results. The maximum titre per dose remains unchanged. The stability of the vaccine in liquid nitrogen also remains unchanged. .

3.2. Choice of the antigen content

The minimum guaranteed dose of vaccine is 1,000 PFU (3.00 $\log_{10}$ Plaque Forming Units) and has been selected based on the results of challenge study in chicks. The maximum release dose of the Vaxxitek HVT + IBD vaccine was selected on the basis of the results of safety studies in chicks.

The stability study shows a good stability of the vaccine. No loss is observed. This was expected as liquid nitrogen storage is known to protect very efficiently the frozen vaccine. An overage is added by the Applicant to a minimum release requirement.

With regard to the specifications, the Applicant has committed to provide a detailed report, based on 10 batches of product.

3.3 Choice of the starting materials of animal origin

The active ingredient is the production-cell associated vHVT013-69 virus vaccine strain, a recombinant turkey Herpesvirus (HVT) expressing the VP2 coding sequence of Infectious Bursal Disease Virus (IBDV). The parental strain of Vaxxitek HVT + IBD (named FC-126) belongs to the serotype 3 of Marek's disease and is widely used in classical vaccination against Marek's disease. HVT strains are apathogenic in all species, and do not replicate in mammalian cells. The inserted sequence corresponds to the sequence coding for the VP2 protein of Infectious Bursal Disease Virus (IBDV). It has been cloned from the 52/70 Faragher strain. The VP2 protein is the only protein of IBDV that induces protection against Gumboro disease. The insertion of the gene coding for the Gumboro VP2 protein in the HVT parental strain allows to prepare a vaccine that can be thus administered to day-old chickens, without presenting any safety problems or immunosuppressive effect.

3.4 Choice of the adjuvant

Not applicable.

II.B. METHOD OF PREPARATION

The formulation of the vaccine is based on a defined volume of active ingredient. The consistency of the formulation is also ensured through the number of cells in the final product.

Formulation data on three batches were presented. Appropriate studies have been validated for the finished product.

II.C. STARTING MATERIALS

1. Listed in a Pharmacopoeia

Dimethyl sulfoxide
Dipotassium phosphate
Gentamycin sulphate
Potassium dihydrogen phosphate
Purified water
Sodium hydrogen carbonate
Sodium hydroxide
SPF embryonated hen eggs
Sucrose
Water for injection
Nitrile elastomer closure
Glass containers for pharmaceutical use
Polypropylene for containers for parental use
Polyvinylchloride

Certificates of analysis were presented.

2. Not listed in a Pharmacopoeia

2.1 Starting materials of biological origin

The starting materials of biological origin entering into the composition of the vaccine are listed below:

Active ingredient: vHVT013-69
Bovine serum
Casein hydrolysate
Fœtal calf serum
Calf serum
Pronase
SPF chicken embryo cells
Trypsin
Tryptose Phosphate Broth (TPB)

Active ingredient: origin and construction of the vaccinal strain vHVT013-69

The virus vector is HVT FC-126, a Herpesvirus from turkeys which is widely used in conventional vaccines against Marek's disease. Using a donor plasmid, the expression cassette (the promotor and cDNA from the VP2 gene of Infectious Bursal Disease Virus and a poly-A sequence) was inserted. The cDNA was made from genomic RNA from virions isolated from the Bursa of chickens

experimentally inoculated with the Faragher 52/70 strain: the sequence matched the published sequence. The locus of insertion was defined. The insertion was targeted through plasmid sequences that were homologous to HVT FC-126 sequences of the virus. Transfection was done in Chicken Embryo Cells co-infected with the parental virus and the plasmid.

The conformity of the construction was checked by PCR and Southern Blot analysis of the recombined region. The correct sequence of the inserted gene and the surrounding nucleotides was verified. The virus stock was verified for absence of parental virus by PCR and immunostaining: no plaques negative for VP2 were detected. The stability of the construction has also been verified: along passages in chicken embryo fibroblasts and along passages in chickens; by sequencing the insertion locus of the virus passaged in chickens and by phenotypic stability assessment after passages in Chicken Embryo Fibroblasts.

Active ingredient: production

Production of the active ingredient is based on a classical seed-lot system: the Master Seed Virus (MSV) was constituted from the cloned vaccinal strain. The control tests performed on the MSV were described. The control tests performed on the Working Seed Virus (WSV) were provided. Tests were also carried out for bacterial and fungal sterility, mycoplasmic sterility, identity and infective titre. The maximal number of passages for preparation of the active ingredient from the MSV is 5. The production flow chart and data from three batches were presented.

Examples of preparation of three batches

The reproducibility of the compliance with the preparation process was demonstrated by the production parameters of three batches of active ingredient.

The control tests document conformity with current Ph Eur monographs and EU guidelines. The viral safety of the WSV is adequately assured by Good Manufacturing Practice (GMP) and by testing of the Master Cell Seed and of the raw materials.

Media

During each production run a roller bottle is kept for sterility control. The culture medium (with the addition of gentamycin, foetal calf serum and calf serum) is sterilised by filtration rather than by autoclaving in order to preserve vitamins and growths factors essential for virus growth.

COMPLIANCE WITH THE EUROPEAN NOTE FOR GUIDANCE FOR MINIMISING THE RISK OF TRANSMITTING ANIMAL SPONGIFORM ENCEPHALOPATHY AGENTS VIA VETERINARY MEDICINAL PRODUCTS.

Certificates of analysis have been presented for all substances of animal origin used.

Bovine, Foetal and calf serum

These starting materials are irradiated using a validated procedure and controlled in accordance with the guidance “General Requirements for the production and control of live mammalian bacterial and viral vaccines for veterinary use” (III/3182/92-EN). The BSE risk is prevented by geographical origin.

Casein hydrolysate

This starting material is irradiated and controlled in accordance with III/3182/92-EN. The BSE risk is prevented by the nature of the substance (milk derivative) and by geographical origin.

Pronase

This starting material is obtained by bacterial fermentation. It is controlled in accordance with III/3182/92-EN.

SPF chicken embryo cells

See Part II.C.

Porcine trypsin

This starting material is irradiated and controlled in accordance with III/3182/92-EN.

Tryptose phosphate broth

This starting material is irradiated using a validated procedure and controlled in accordance with III/3182/92-EN. The BSE risk is prevented by geographical origin.

Conclusion on TSE risk

The ruminant raw materials used in the in the preparation of the final product comply with the current regulatory texts related to the TSE guideline (EMEA/410/01 – Rev.1) and Commission Directive 1999/104/EEC.

2.2 Starting materials not in a pharmacopoeia – Starting materials of non-biological origin

Adequate documentation was provided.

II.D. CONTROL TESTS DURING PRODUCTION

Production was detailed in flow charts. Given that the active ingredient is directly used to blend the vaccine without storage, no in-process control tests are performed on it. The values of the main parameters were provided. Sterilisation steps are performed in accordance with Ph. Eur. 5.1.1. The quality of the vaccine is thus ensured during its manufacturing at the industrial scale.

The specifications/acceptance limits of the in-process tests were adequately described.

II.E. CONTROL TESTS ON THE FINISHED PRODUCT

These tests are summarised below.

Control tests on the frozen viral suspension:

These include: Appearance, pH, Volume, Identification, Assay, Safety test, Bacterial and fungal sterility, Mycoplasmic sterility, Viral purity, Extraneous agents on avian cell cultures and Extraneous agents in SPF chickens.

Control tests on the diluent include:

Appearance, pH, Volume, Compatibility and Bacterial and fungal sterility.

The full range of tests for extraneous agents will be carried out on the final product (on 10 batches). The results obtained will be presented to justify limited testing in the framework of variation of the authorisation.

Compared to the Vaxxitek HVT + IBD vaccine already authorised in EU, only the minimum release titre has been increased. There is no other change compared to the authorised product (frozen vaccine and diluent). The quality control tests performed and techniques used are identical to those approved for the already authorised Vaxxitek HVT + IBD product.

II.F. STABILITY

1. Stability of the active substance – see section II.B

2. Stability of the finished product

Frozen suspension

With regard to both biological and physicochemical stability, satisfactory data have been provided to justify a shelf-life for the non-reconstituted vaccine of 36 months at –196°C.

Diluent

Satisfactory accelerated stability data are presented on one batch of diluent stored for 6-9 months at different temperatures and different relative humidity conditions. The diluent remains stable after 12 months storage at 30 +/- 2°C in polypropylene bottles or polyvinyl bags. The recommended storage temperature of the diluent was 30°C and, as a precaution, the statement "do not freeze" was included in the SPC.

3. Stability of the Reconstitutued Product

Satisfactory results have been presented using four batches of diluent in bottles to reconstitute one batch of thawed suspension then stored at room temperature (approximately 20°C). All results are in accordance with the Specific Requirements for the Production and Control of Avian Live and Inactivated Viral and Bacterial vaccines, i.e. the loss in titre over 2 hours was not more than 50% of the original titre if measured by PFU test (i.e. not more than 0.3 log).

The stability for the vaccines and diluent for the extension application were identical to those for Vaxxitek HVT + IBD as already authorised. It was considered that there was no impact on the quality of the vaccine during its shelf-life. Therefore, with the overage added to the minimum titre, this can be considered acceptable.

Confirmation was provided that out of specification batches would not be released. The quality of batches complying with the *in ovo* specifications after manufacturing is thus ensured throughout the whole period of validity of the vaccine.

II.H. GENETICALLY MODIFIED ORGANISMS

The active ingredient is a recombinant Herpesvirus of turkeys (HVT), the genome of which contains the VP2 gene of IBDV. The resulting strain is named vHVT013-69. This strain was obtained by recombination between the FC126 strain of HVT and isolated sequences of the 52/70 Faragher strain of IBDV.

In order to evaluate the risk related to GMOs, it is necessary to study the interaction between the GMO and the environment. In particular, the risks of spread from the vaccinated animal must be analysed by studying the likelihood of spread to other animals either directly or indirectly, the consequences of a possible spread and how to control these risks. The dossier submitted analysed these different issues and followed the recommended format. The construction of vHVT013-69 as well as its stability were evaluated under the analytical section.

Despite the fact that the use of a recombinant replicative strain in a non-natural target species could be in principle questionable in view of the potential risks associated with the adaptation of a virus to a new host, in this particular case, the parental strain FC-126 from which the recombinant virus is derived has been widely used as a vaccine. The foundation of the risk analysis for this product can,

therefore, be centred on the analysis of the identity and/or differences of the biological properties between the recombinant and the parental strain. More precisely, if the recombinant strain is identical to the parental strain for the two types of criterion that are related to safety a) the dissemination in the body of vaccinated animals and the lack of clinical signs following experimental administration of several species including the target species and b) the diffusion to contact animals and in the environment, the risk associated with the recombinant, if any, should be in the same order of range than that of the parental vaccine strain.

1. Pertinent characteristics of the recombinant vaccine related to the risk analysis

The recombinant vaccine strain named vHVT013-69 has been constructed following a protocol which was provided. The region of insertion was targeted through homologous recombination between the DNA of the parental FC-126 strain of HVT and a plasmid harbouring the gene of interest surrounded by homology arms. The two DNAs were transfected in Chicken Embryo Cells, the resulting bulk of virus was purified and plaques corresponding to the recombinant virus were identified.

The conformity of the construction was checked by Polymerase Chain Reaction (PCR) and Southern-Blot. Stock used to constitute the MSV was checked by PCR analysis and by immunostaining of several hundred of plaques for the expression of the transgene without evidence of any negative plaques which could correspond to the parental virus. Its stability along passages beyond the maximum level used in production was evaluated by the same techniques. The genomic stability of the inserted and surrounding sequences were confirmed by sequencing the insertion locus of the virus passaged in chickens.

The main risks associated with the recombination process have been avoided. The analysis of the phenotype of the resulting recombinant strain is nevertheless essential to evaluate the risk associated with the construction, as developed below.

2. Comparison of the behaviour of the parental and recombinant trains following administration to target and non-target species

The different potential risks associated with the use of the vaccine were evaluated.

2.1 Vaccination of chickens

2.1.a Clinical safety

The safety of the recombinant strain was evaluated after inoculation of an overdose by the SC route in 1-2 day-old chickens. No sign of Marek's disease nor specific gross lesions at necropsy were recorded. The results for the recombinant strain were in line with the known properties of the HVT strains in chickens, and showed that the recombination event did not lead to major modifications of the clinical safety of the parental strain.

2.1.b Risks of spread

The same pattern of dissemination in the body of chickens experimentally infected by the subcutaneous route was shown for both the parental and the recombinant strain. In both cases, the virus could be isolated only in feather follicles, and not from cloacal swabs, tracheal swabs and bursa of Fabricius, despite the fact that higher doses than that used for vaccination were used. The two strains did not show any difference related to their dissemination potential in the conditions of this assay. The lack of isolation of the recombinant strain from the bursa of Fabricius excludes a potential modification of the viral tropism to this organ due to the insertion of the VP2 IBDV gene. The lack of virus isolation from the tracheal and cloacal swabs suggests that the transmission of both strains by the respiratory or faecal routes is of low probability. The persistence of infection was comparable for both strains.

The horizontal spread from vaccinated to naïve chickens was also analysed. Chickens maintained in contact with chickens vaccinated with overdoses of both strains remained negative. The horizontal spread from vaccinated chickens to turkeys was also evaluated. Naïve turkeys were reared in contact with chickens vaccinated with an overdose of either the parental or the recombinant strain. Examination demonstrated that both strains were transmitted to turkeys in these conditions. No significant difference between strains was evident.

2.2 Accidental spread to Turkeys

2.2.a. Clinical safety

An overdose of the parental and the recombinant strains was tested in turkeys. No difference between the two strains was recorded. The groups vaccinated with both strains showed growth performances comparable to those of the control non-inoculated group and did not show any evidence of local or general lesions at necropsy.

2.2.b. Risks of spread

The horizontal spread from Vaxxitek HVT + IBD-infected turkeys to naive turkeys was evaluated. The recombinant strain was able to spread from infected turkeys to naïve turkeys, a species in which it was demonstrated to be as safe as the parental strain. In the presence of other animals infected with an HVT field strain, the naïve turkeys seem to be infected more rapidly by the field strain. On the other hand, in HVT-infected turkeys, Vaxxitek HVT + IBD does not seem to spread easily, probably because of the pre-existing immune response. It is likely that the spread of the recombinant strain would be limited in turkey populations that are very frequently infected by HVT.

2.3 Accidental spread to other species

Information was provided on the safety of the recombinant strain for other bird species: pigeons, pheasants, ducks, quails and partridges. In all cases inoculation of an overdose of Vaxxitek HVT + IBD did not lead to any clinical sign or gross lesion.

The parental and the recombinant strains were tested in mice and guinea pigs as models of mammalian species. No death, general or local signs were evident and the growth performances of each group were similar to that of the control group. Because the biological properties of Vaxxitek HVT + IBD do not differ from the parental strain, no additional specific risk for humans is anticipated.

3. Risks associated with recombination of the recombinant vaccine strain

In all the hypotheses tested, the analysis demonstrates a low to very low likelihood of hazard and very insignificant clinical and epidemiological consequences. The only concern is the low risk of emergence of a MDV or wild-type HVT strain expressing IBDV but without clinical consequences or true difficulties in antibody screening against IBDV.

The data supplied by the manufacturer for a risk analysis and complemented by available scientific data are based on the fact that a) the recombinant strain is derived from a widely used vaccine strain and b) no modification of the biological properties of the strain could be shown nor foreseen.

It can be considered that Vaxxitek HVT + IBD does not show any documented clinical or epidemiological safety risk. Moreover, based on current scientific knowledge and virological concepts, no foreseeable risk associated to its use under the conditions described was identified.

III. SAFETY ASSESSMENT

III.A. INTRODUCTION

In order to demonstrate the safety of the Vaxxitek HVT + IBD vaccine, tests were carried out both in the laboratory and in the field. The tests were carried out in compliance with the requirements in force at the time and enabled the assessment of possible risks associated with Vaxxitek HVT + IBD under the claimed conditions of use. The requirements, concerning live vaccines, of Directive 81/852/EEC, amended by Directive 92/18/EEC, Part 7, (now codified as Directive 2001/82/EC of the European Parliament and of the Council) have been fulfilled through the performances of tests. The European Pharmacopoeia monographs were used as a guide (Vaccinum bursitidis infectivae aviariae cryodesiccatum, 1997:0587; Vaccinum morbi marek vivum, 1997:0589).

Ecotoxicity tests were carried out in different avian susceptible species, especially in turkeys, (the natural host of the HVT (Herpesvirus of Turkey virus). During the development of the product, scientific advice was provided by the CVMP. Since the vaccine contains a live recombinant virus, the described laboratory trials were concerned by Council Directive 90/219/EEC. Appropriate authorisation was obtained in France and the clinical trials carried out involved a deliberate release into the environment under the terms of Council Directive 90/220/EEC.

III.B. GENERAL REQUIREMENTS

All the safety tests were carried out in the category of the target species (i.e. one-day-old chickens). A volume of 0.2 ml was administered (claimed quantity for one dose of product). The content of live virus (vHVT013-69) was generally the maximum release dose (as required in European Guidelines for production and control of avian live vaccine). Lower dosages were used in specific studies, and a justification was given in respective sections. The vaccines batches used for safety testing were produced according to the manufacturing process described in the quality part of this application.

The safety test was carried out in the claimed category of the target species, (*i.e.* the chicken eggs at 18 days of embryonation) for which Vaxxitek HVT + IBD vaccine is intended. The recommended volume of 0.05 ml was administered. The content of live virus (vHVT013-69) was at the maximum dose. The vaccine batch used for safety testing was produced according to the manufacturing process described in the quality part of this application.

The safety of the administration of an overdose and of a repeated dose of vaccine have been demonstrated using the S.C. route. The S.C. route was also chosen as the representative route for the demonstration of the safety regarding the following aspects: examination of reproductive performances, impact on immunological functions, interactions, dissemination and spread in the target species, biological properties (genomic stability, recombination risk), ecotoxicity and safety in non-target species, safety in field conditions.

ABBREVIATIONS

HVT	Herpesvirus of turkeys
IBD	Infectious Bursal Disease (Gumboro Disease)
IBDV	Infectious Bursal Disease Virus
MD	Marek's Disease
MDV	Marek's Disease Virus
MDA	Maternally derived antibodies
PFU	Plaque Forming Unit
MSV	Master Seed Virus (vHVT013-69)
HVT FC-126	Parental non-recombinant strain
SPF	Specific Pathogen Free
DMSO	Dimethyl-Sulfoxide

III.C. LABORATORY TESTS

All laboratory studies were conducted according to Good Laboratory Practice (GLP) and used SPF chickens.

1. Safety of the administration of one dose

With regard to the HVT vector, the safety of the administration of one dose and of an overdose was studied according with requirements of the European Pharmacopoeia monograph for live vaccines against Marek's disease (1997:0589). With regard to the recombinant nature of the vaccine, another study was also presented demonstrating the safety of the administration of one dose of the vaccine strain, with respect to the Gumboro disease criteria (monograph 1997:0587).

Study to assess the safety of the administration of one dose of the vaccine strain in SPF chickens with respect to Gumboro disease criteria according to requirements of European Pharmacopoeia monograph (1997:0587, Avian infectious bursal disease live vaccine).

Groups of sixteen SPF one-day-old chickens were constituted: G1 inoculated with one dose of vaccine strain; G2 inoculated with an overdose of vaccine strain; G4 inoculated with sterile diluent acting as control group; and G5 was inoculated with an overdose of the vaccine strain without DMSO.

Complete examinations were carried out, including daily clinical observations (general and local signs) during 21 days after administration of the products, follow-up of growth by weighing all the animals, and post mortem examinations.

Serological analyses were also carried out to check the vaccine response by measuring specific antibodies against infectious bursal disease virus and specific antibodies against Marek's disease virus.

The results are summarised as follows:

Clinical: there were no general reactions or injection site reactions.
Mortality: no case of mortality attributable to the vaccine occurred in any group.
Body-weight: growth was the same in both groups during the period of observation.
Necropsy: none of the chickens showed lesions of the bursa of Fabricius.
Serology: the serological results provide evidence for seroconversion in groups G1, G2 and G5.

The study complied with the requirements of European Pharmacopoeia monograph on Avian infectious bursal disease live vaccine (1997:0587).

- **Vaxxitek HVT + IBD – Recombinant live vaccine vHVT013-69 – Safety in chickens after *in ovo* vaccination.**

Objective: to assess the safety of Vaxxitek HVT + IBD vaccine in SPF chickens after *in ovo* vaccination, according to the requirements of the European Pharmacopoeia monographs (1997:0587 for live vaccine against Gumboro disease, and 1997:0589 for live vaccine against Marek's disease), and in comparison with a reference HVT vaccine .

Methodology:

- four randomly selected groups of 50 SPF 18-day old embryonated eggs were set up on D-4 and inoculated manually as follows:
  - G1 : Vaxxitek HVT + IBD vaccine (50 µl),
  - G2 : Reference HVT vaccine,
  - G3 : sterile diluent (50 µl),
  - G4 : non-inoculated controls.
- On D0, the chicks were weighed. On D3, group were sizes of 35.

Results:
High hatchability rates (90% to 100%) were found in all groups, with no statistically significant differences between the groups. Viability was 100% in the 3 inoculated groups and 98% in the controls.

- There was no statistically significant difference between the mean body weights gain of the 4 groups during the study.
- Anti-HVT antibodies were found in 9 out of 10 chickens in Vaxxitek HVT + IBD and in 7 out of 10 in reference HVT vaccine. The unvaccinated controls, were all seronegative.
- No sick or dead animals were recorded during the study. Post-mortem examination on D21 did not reveal any macroscopic lesions on the bursa of Fabricius. On D42, no macroscopic lesions of Marek's disease were found in any of the animals.

histological examination was carried out on the bursa of Fabricius on D21.
From data from field trials and considering the number of chicks to the number of eggs as "hatchability", the percentages of hatching are higher in vaccinates than in controls. Therefore it can be considered that the vaccine has no negative effect on hatchability. No significant lesions related to Gumboro's disease and Marek's disease were recorded.

In conclusion the safety of the vaccine when administered *in ovo* at 18 days of incubation was therefore considered acceptable.

2. Safety of an administration of an overdose

Study to investigate the safety of the Vaxxitek HVT + IBD vaccine and the vaccine strain in SPF chickens with respect to Marek's disease, in accordance with the requirements of the European Pharmacopoeia monograph (1997:0589).

Groups of 40 chicks aged one day were set up on D0: G1 non-inoculated controls; G2a inoculated with an overdose of the vaccine strain; G2b inoculated with an overdose of Vaxxitek HVT + IBD vaccine. Groups were assessed clinically and checked for microscopic lesions consistent with MD.

In vaccinated groups, the number of surviving animals at the end of the trial was equal to that observed in controls (G3), and none showed macroscopic lesions of Marek's disease. No histological lesion consistent with MD was observed in these groups.

The safety of Vaxxitek HVT + IBD with respect to Marek's disease was demonstrated with regard to the requirements of the European Pharmacopoeia monograph (1997:0589). As the safety of the administration of an overdose of vaccine was demonstrated in this test, the safety of the administration of one dose was also demonstrated.

3. Safety of the repeated administration of one dose.

Study to assess the safety of a repeated administration of one dose of Vaxxitek HVT + IBD vaccine in SPF chickens, with respect to Gumboro disease and Marek's disease criteria.

Two groups of 20 SPF day-old chicks were set up on D0 and injected as follows: G1: one dose of the vaccine on D0, then on D14; G2: sterile diluent for Marek's vaccines on D0, then on D14.

Clinical follow-up was carried out daily during the study in particular for signs of Gumboro disease and Marek's disease. All the animals were weighed on D0. On D56, all the birds were necropsied for signs of specific lesions of Gumboro or Marek's disease. The repeated injection of Vaxxitek HVT + IBD had no influence on the body weight gain from D0 to D56. No sick or dead animals were recorded in the vaccinates throughout the trial. On D56, no clinical or necropsy lesions consistent with Marek's or Gumboro disease were noted in any bird.

The safety of a repeated administration of the maximum dose of the vaccine Vaxxitek HVT + IBD was therefore established.

4. Examination of reproductive performance

Study to assess the safety of the Vaxxitek HVT + IBD vaccine in laying pullets.

Safety was assessed by clinical follow-up and monitoring of the lay until 38 weeks of age. Three groups of SPF chickens were set up as follows: G0: 10 animals used as serological controls; G1:153 birds vaccinated with one dose of the vaccine at day-old; G2:152 birds used as unvaccinated controls. The 2 last groups were reared in the same animal house until the age of 38 weeks.

Mortality was recorded, lay monitored and serological testing carried out. The serological results validated the SPF status of the animals on D0. The booster effect of the injection of inactivated vaccine was also confirmed in G1. Moreover, the serological negativity of the sera from G2 (controls) at 10 weeks of age showed that Vaxxitek HVT + IBD vaccine did not spread from vaccinated birds to non-vaccinated ones.

With regard to mortality, the viability percentages were satisfactory and similar in the 2 groups, showing the absence of adverse effect of this vaccine used at high dose in pullets. The laying performances were also the same in the 2 groups, indicating that Vaxxitek HVT + IBD used at high dose is harmless for the lay.

Safety with regard to reproductive functions in breeder chickens after subcutaneous vaccination

This trial studied the safety of Vaxxitek HVT + IBD vaccine regarding reproductive functions in field condition using 461 females and 60 males from the same farm. Fifteen one-day old female chicks were chosen before vaccination on D0, to be used as serological controls. Two groups were set up with the remaining chicks with one group receiving other propriatory vaccine for MD and/or HVT. The two groups also received a classical vaccination programme for future breeders before the start of lay, including IBD vaccination.

Data regarding safety of the vaccine for general condition of the birds (mortality and weights) were similar in the two groups.

NB: During the 36th week of age, there was a failure in the feeding system resulting in a decrease of performances. For this reason, only the data of lay and viability collected until 35 weeks of age were developed.

The vaccine is unlikely to affect male fertility. A warning has been included in the SPC that “in the absence of data, the vaccine should not be used in breeding birds and birds in lay”.

5. Examination of immunological functions

The impact of Vaxxitek HVT + IBD vaccine at a maximum dose upon immunological functions after vaccination, using the model of interference with the immunisation against Newcastle disease (ND) was investigated. The Newcastle challenge model is classically used to reveal an immunodepression, affecting either humoral immunity (serology criteria) and global protection (protection criteria).

Protection against Newcastle Disease has been demonstrated by a challenge with a dose of Newcastle Disease Virus (NDV) that induced 100% mortality. It was therefore, concluded that the vaccine does not interfere with immunisation against Newcastle disease.

6. Special requirements for live vaccines

6.1. Spread of the vaccine strain

The spread of the vaccine strain in the target species was presented. The spread in turkeys was also studied and results evaluated in section III.E. Ecotoxicity.

The spread of the vaccine strain from chicken to chicken, in comparison with the spread of the parental HVT FC-126 non-recombinant strain was evaluated.

Three groups of six SPF, day-old chicks were constituted: G1 was inoculated with the vaccine strain, G2 was inoculated with the parental HVT and G3 injected with diluent for Marek's vaccine. Each group was placed in an isolating unit in contact with 4 non-inoculated chickens of the same origin and age.

The viruses were tested for in the leukocytes of the birds by means of isolation tests on chicken embryo cell cultures. Test were also conducted to detect the presence of specific antibodies to HVT and IBDV viruses. The virus was found in both inoculated groups. All the individual samples from contact or control (injected with diluent) birds remained negative. HVT and IBDV antibodies were detected in the inoculated birds while all contact or control birds remained seronegative.

Additional data was provided to show that spreading of vaccine virus from chicken to chicken does not occur as there is very low viral excretion and because oral, ocular and nasal routes of contamination with the vaccine strain are not effective in chickens.

Comparison of the vaccine infectivity in turkeys and in chickens – Dose response relationship.

This study was set up to compare the infectivity of the vaccine in turkeys and in chickens when administered by non-parenteral route or by subcutaneous route. On D0, 8-day-old conventional turkeys and 6-day-old SPF chickens were inoculated with increasing doses of vaccine (by a non-parenteral route except one group). The presence of anti-IBDV antibodies and antibodies against HVT was checked.

The results showed that both chickens and turkeys were infected by the vaccine with a low dose inoculated by subcutaneous route; chickens were not infected by the vaccine when inoculated by non-parenteral route whereas turkeys were partially infected after inoculation by non-parenteral route of a high dose of vaccine only. These results could explain the absence of spread of the vaccine to chickens and the possible spread to turkeys. Turkeys are more susceptible to infection by non-parenteral route than chickens, but very high doses are necessary to induce a partial seroconversion.

Spread of the vaccine virus and of the parental strain in the environment – Search for viruses associated with dust and litter after inoculation of SPF chickens.

The purpose of this trial was to assess the infectious titre of Vaxxitek HVT + IBD vaccine virus and of the HVT parental strain in dust and litter excreted by inoculated SPF chickens. Three groups named G1, G2 and G3 of 15 one-day-old SPF chicks were set up on D0 and inoculated subcutaneously as follows: G1: Vaccine virus; G2: HVT FC126; G3: diluent.

Each group was settled in separate isolators. Dust and litter from each isolator were sampled and inoculated into chicken embryo cells cultures. In the conditions of the study, no virus was isolated. If vaccine virus or the HVT parental virus were present in dust and litter excreted by inoculated chickens, their level of re-excretion was too low to allow their re-isolation.

The final objective of these studies was to demonstrate that oral/spray route is not efficient for infection in chickens. These results also confirm the common knowledge on inefficiency of Marek vaccination by non parenteral routes. Chickens have a lower sensitivity to HVT virus than turkeys.

These new studies suggest that spreading of vaccine virus from chickens to chickens does not occur because there is very low virus excretion and because oral, ocular and nasal routes of contamination with the vaccinal strain are not effective in chickens.

6.2 Dissemination in the vaccinated animal

Evaluation of the dissemination and the tissue tropism of the vaccine strain in the body of vaccinated chickens, and to compare it with the parental HVT strain dissemination.

Seventy day-old SPF chickens were distributed in four groups: G1 vaccinated with the vaccinal strain; G2 vaccinated with the parental HVT non-recombinant strain; G3 inoculated with the diluent for Marek's vaccine and G4 challenged with the virulent MDV Rb-1b strain.

The bursa of Fabricius was sampled and treated for virological research. Feather samples, in order to search the virus in the feather follicles were also taken.

Tracheal and cloacal swabs were regularly performed. At the end of the study, each living animal of groups G1, G2 and G3, was blood sampled for evaluation of a specific serological response.

All cloacal and tracheal swabs were negative (first and second passage), as well as all bursae of Fabricius. Marek's virus was identified in feather follicles, in G1 and G2 during all stages of the observation period. It was also detected in G4 at the first sampling date. A serological response against HVT virus was detected in all animals of G1 and G2 groups.

These results confirm the known viral distribution of HVT in the body of vaccinated chickens. They establish also that the parent and the recombinant HVT multiply in the follicles of growing feathers, the known predilection site for replication of HVT in the host.

6.3 Reversion to virulence of attenuated vaccines

6.3.1 Serial passages of Vaxxitek HVT + IBD vaccine in the target animal

A study was carried out to obtain a strain resulting from the vaccine strain after passages on chickens. This experiment was carried out as part of the study of reversion to virulence.

G1: 10 day-old chickens were intramuscularly administered with the vaccine strain. Seven days later, a blood sample was taken from each and the leukocytes isolated and pooled. G2: 10 day-old chickens were inoculated with part of the leukocytes suspension by the intraperitoneal route in order to carry out a second passage of virus on birds. Another part of the leukocyte suspension was stored in liquid nitrogen. The above operations were repeated on the same type of birds. The last passage was carried out on 20 chicks from which blood was sampled at the age of 14 days. Neither clinical sign nor mortality were observed during the period of observation of the birds (one or two weeks). The virus was detected at each passage by isolation.

Whilst the test performed is not in conformity with the European Pharmacopoeia, this is acceptable as the irreversibility of attenuation test is not required for this product.

6.3.2 Reversion to virulence – Safety of passages of Vaxxitek HVT + IBD

In this section, only the results relative to the reversion to virulence study are presented.

A study was carried out to evaluate the safety in SPF chickens of the strain obtained after being passaged in chickens, according with the requirements of the European Pharmacopoeia monograph for live vaccines against Marek's disease (1997:0589).

Five groups of 40 chicks aged one day were set up on D0: G1: non-inoculated controls, G2a: animals subcutaneously inoculated with the vaccine strain; G2c: animals subcutaneously inoculated with the

passaged strain of the vaccine strain in chickens; G2d: animals subcutaneously inoculated with the passaged strain amplified by 4 passages in chicken embryo cells (CEC); G3: non-inoculated controls, non-challenged.

Groups (G1, G2 groups and G3) were monitored clinically. At the end of the trial, all surviving animals were euthanised and necropsied for signs of MD. On D28, five animals in each G2 group and in G3 were blood sampled for anti-IBDV antibodies and on D120, 10 animals in each G2 group, and all animals in G3 were blood sampled: anti-HVT antibodies were looked for in individual sera.

The high rate of challenged controls (G1) which either died or showed macroscopic lesions of MD after challenge was evidence of the susceptibility of the animals used.

Because of the uncertainty of the viral inoculation of this passaged strain, the group inoculated with the amplified passaged strain (G2d) was therefore more relevant for an evaluation of the reversion to virulence potential. All animals in this group displayed anti-HVT and anti-IBDV antibodies, which validated the viral take. None of the surviving animals in G2d showed any macroscopic lesions of MD.

Histological results in the groups which received the passaged virus (amplified or not) were not statistically significantly different from those in the group inoculated with the unpassaged virus. No histological lesions suggestive of Marek's disease were observed in the different groups, in any organ sampled.

6.4 Biological properties of the vaccine strain

6.4.1 Replication particularities

Study to evaluate the persistence of the Vaxxitek HVT + IBD vaccine and the evolution of Gumboro antibody titres in vaccinated SPF chickens, in comparison with the parental HVT non-recombinant strain.

Forty day-old chickens were distributed in four groups: G0: intial serological controls, tested serologically; G1: animals vaccinated with Vaxxitek HVT + IBD vaccine; G2: animals inoculated with 3.0 log10 PFU of the parental HVT strain; G3: controls, non-inoculated. On D28, D42 and D61, all chickens from G1, G2 and G3 were blood sampled. The leukocytes were isolated from each sample, and inoculated to chicken embryo cell cultures. On D61 samples, the number of PFU was counted on cell cultures to quantify the viral load.

A serological monitoring was also performed: all animals from G0 were tested on D0, and blood sampling was carried out on the animals from G1 and G3 on D21, D42 and D61. All sera were tested for anti-IBDV neutralising antibodies.

Virus isolation

The presence of virus was observed in most inoculated animals on D28, and in all of them afterwards (on D42 and D61). As expected, no virus was isolated in controls. The quantification of PFU in inoculated groups showed that the viral charge was about 4 times as important in G2 as in G1 on D61.

Serology

Animals from G0 proved to be all negative for anti-IBDV antibodies on D0. Control group G3 as well tested negative during the whole study. In G1, all the sera tested were positive as early as D21, and the titres increased regularly until the end of the trial.

Under the conditions of the trial, persistence of HVT virus in SPF chickens was demonstrated during at least 8 weeks, for the parental and recombinant virus as well. Yet, the trial evidenced the same or

slightly diminished viral load in animals vaccinated with Vaxxitek HVT + IBD, as compared with animals inoculated with HVT parental strain.

6.4.2 Safety for various species

See Part III.E.

6.5 Recombination or genomic reassortment of strains

Study to assess clinically the recombination risk between Vaxxitek HVT + IBD vaccine with others Marek's disease virus (MDV) serotypes 1 and 2.

Three MD viruses of variable pathogenicity were used: Strain GA22: serotype 1, virulent; Strain SB-1: serotype 2, avirulent; Strain Rispens: serotype 1, avirulent. Eight groups containing at least 50 SPF day-old chicks were set up on D0 and inoculated as follows: G1: Vaxxitek HVT + IBD vaccine combined with virulent strain GA22; G2: Vaxxitek HVT + IBD vaccine combined with strain SB-1; G3: Vaxxitek HVT + IBD vaccine alone; G4:strain GA22 alone; G5: strain SB-1 alone; G6: Vaxxitek HVT + IBD vaccine combined with strain Rispens; G7: strain Rispens alone; G8: non-inoculated hatchmates.

On D0, 10 chicks from the same flock were sampled as serological controls. On D120, ten animals in each group were blood sampled and tested for Marek's disease antibodies. All the birds were weighed on D120. Clinical examination was carried out daily for 120 days. Sick or dead animals were recorded, and any dead or euthanised bird was necropsied for signs of Marek's disease, then sampled (spleen, liver, kidneys and nerves) for subsequent histology. On D120, all the surviving animals were euthanised and necropsied for signs of Marek's disease. At this date, 10 randomly selected birds in G1 and G4 were also sampled for histology.

No adverse effect of the combination of Vaxxitek HVT + IBD with any of the MD viruses upon body-weights, in comparison with the viruses inoculated alone, was observed on D120 (statistical evaluation). The highest mortality rates were recorded in G1 and G4, which had received the mild pathogenic GA22 strain. Cumulative mortality curves for G1 and G4 also show that MD is delayed in groups inoculated with both GA22 and Vaxxitek HVT + IBD strains. Most dead animals in groups G1 and G4 showed macroscopic and histological lesions consistent with Marek's disease. At final necropsy, 4 animals in each G1 and G4 showed grossly observable lesions consistent with Marek's disease. No typical lesions of Marek's disease were found in any other animal. In the other groups none of the dead animals in these groups showed any gross lesions of MD and no surviving animals on D120 showed any gross lesions of MD at the final necropsy.

Serology

Serological results showed no antibodies against Marek's disease virus on D0. On D120, MD antibodies were found in all sera from inoculated groups. There was no difference in pathogenicity of any of the MD viruses strain when combined with Vaxxitek HVT + IBD.

7. Study of residues

The product does not contain any adjuvant. The excipients Sodium hydrogen carbonate, dimethyl sulfoxide, sodium hydroxide, hydrochloric acid, dipotassium phosphate and potassium dihydrogen phosphate are included into Annex II of Council Regulation 2377/90/EEC. For F10 HAM Medium, 199 medium and phenol red, the data provided by the Applicant were assessed and the substances considered not to be pharmacologically active at the doses used. Casein hydrolysate and water for injection are not considered as falling within the scope of Council Regulation (EEC) No. 2377/90. It can be concluded that no residue exposure is expected and that a withdrawal period of 0 days is justified.

8. Interactions

Study to assess the safety of Vaxxitek HVT + IBD vaccine and its interaction a live vaccine against Marek's disease commonly used in layer breeds (CRYOMAREX Rispens).

Methodology:

Two groups were defined as follows: G1 contained birds subcutaneously vaccinated with CRYOMAREX Rispens at day-old; G2 contained birds vaccinated CRYOMAREX Rispens combined with the Vaxxitek HVT + IBD vaccine, at day-old.

During the rearing phase, both G1 and G2 received a complete vaccination programme with live vaccines, including infectious bronchitis, Newcastle disease, avian encephalomyelitis and swollen head syndrome vaccines. At 120 days of age (i.e. 120 days post-vaccination with CRYOMAREX Rispens alone or combined with Vaxxitek HVT + IBD), 44 birds in each G1 and G2 were euthanised and necropsied for signs of Marek's disease: hypertrophy and/or tumour formations on main organs, and hypertrophy of the nerve plexuses were looked for. Histology analysis of several bursae of Fabricius (BF) was also carried out as an additional safety criterion.

Results:

No lesions evocative of Marek's disease were found in any of the birds in the group which received CRYOMAREX Rispens only, nor in the group which was administered CRYOMAREX Rispens associated to Vaxxitek HVT + IBD. A heterogeneous appearance of the bursae of Fabricius was noted in both groups. Histology examination of these organs allowed to check that these differences were non-specific. The simultaneous administration of Vaxxitek HVT + IBD and Rispens vaccines is compatible. No interactions between the two vaccines resulting in undesirable side effects were noted.

Vaxxitek HVT + IBD – Recombinant live vaccine vHVT013-69 – Safety – Adverse effects on immunological functions.

This study was presented in section C.5 (examination of immunological functions). Overall it can be concluded that from a safety point of view, no effect is observed when another live Marek's Disease vaccine and a live Newcastle Disease vaccine is administered simultaneously with the Vaxxitek HVT + IBD vaccine.

In the same usual field conditions of Marek's disease serotype 1, ND and IB vaccination, the situation for concurrent administration with Vaxxitek HVT + IBD *in ovo* would be the following:

- concurrent administration (different time, different route, different site) for Marek's disease vaccines serotype 1
- concurrent administration (different time, different route, different site) for ND and IB vaccines.

The compatibility results provided were relevant to justify the compatibility statement for *in ovo* use of Vaxxitek HVT + IBD at 18 days of embryonation and vaccination with other vaccines 3 days later at the soonest, at day-old.

III.D. FIELD STUDIES

Study to assess the safety of Vaxxitek HVT + IBD in field conditions. The trial was carried out in conventional broilers after subcutaneous vaccination.

Methodology:

368 day-old chicks were divided in two groups of 184 chicks each, defined as follows: G1: non-vaccinated controls, injected with sterile diluent; G2: birds vaccinated with Vaxxitek HVT + IBD. The birds were subcutaneously injected with AccuVac vaccinator. The animals also received a standard

vaccination schedule against infectious bronchitis. Ten other animals were blood sampled for serology on D0, before any vaccination was done. On D28 and D49, a blood sampling was performed on 10 randomly chosen animals per group.

Clinical follow-up was carried out until slaughter on D49. Zootechnical data, such as animals body-weight (per cage) and feed consumption were also regularly recorded. A post-mortem inspection was carried out for each carcass at the farm site on D49.

Results:

The percentages of mortality in the two groups during the trial were the same. No specific lesions were noted throughout the trial and at slaughter, and in particular, no lesions consistent with Marek's or Gumboro disease were seen. The feathering of all birds remained normal all through the trial. The weight gain in the vaccinates and the controls were statistically equivalent at any date. There were also no significant differences between the feed conversion indexes of the two groups for each time periods. The vaccination had no consequences on the zootechnical performances of the broilers.

On D0, IBD antibody levels in the chicks were high. There was a decrease in the antibody titres afterwards in the two groups until D28. A serological conversion was then observed on D49 in the vaccinates, while the antibody level decreased in the controls. The safety of subcutaneous vaccination with Vaxxitek HVT + IBD has therefore been demonstrated in day-old chickens in field conditions.

III.E. ECOTOXITY

Due to the recombinant nature of the vaccine, a specific assessment of safety for the vaccinated animals, for the environment and for the consumer of vaccinated animals is presented in Part II.H. of the dossier (according to Council Directive 90/220/EEC). All genetic, phenotypic, and biologic aspect of the recombined virus, and comparison with the parental virus, are studied with regard to the potential recombination consequences.

The extremely limited host avian range of HVT and IBDV makes it unlikely that other birds species become easily infected and diseased. Toxicity studies have been performed in avian species comprising birds which are phylogenetically closely related or even members of the same order Galliformes, such as the quail, pheasant, partridge, and turkey as a positive reference bird species but also birds of different orders such as the domestic pigeon (order Columbiformes) and the domestic duck (order Anatiformes). In these studies, the mouse and the guinea-pig were also included.

Studies in avian species

The Applicant performed 5 studies in non susceptible species (pheasants, ducks, partridges, quails and pigeon) and two studies in the susceptible species turkeys. All studies were GLP compliant. The design and conduct of each of these experiments was quite similar in each case. The animals were vaccinated with the RMB recombinant vaccine. Unvaccinated animals served as a control. Under the conditions of the trials, the safety of Vaxxitek HVT + IBD vaccine in the investigated species was demonstrated.

Pigeons inoculated with Vaxxitek HVT + IBD vaccine showed either no antibodies or very low antibody titres . Given the high dose administered, it is thus likely that there is no multiplication of the virus in this species.

No turkeys displayed adverse clinical or pathological reactions. No pathological lesions attributable to IBD and MD were noted following the administration of Vaxxitek HVT + IBD vaccine and the parental HVT vaccine.

Spread in turkeys

The likelihood of exposure to the virus could be possible for several weeks, as suggested by the kinetics of dissemination in the body of the vaccine strain. Although the persistence of the vaccine

strain in the environment, no direct contamination amongst chickens was evidenced. The following trials evaluate spread in the most susceptible species.

Study to evaluate the spread of the vaccine strain and, concurrently, of the parental non-recombinant strain, from chickens to turkeys.

Methodology:

Three groups of SPF, day-old chicks were constituted: G1 inoculated with one maximal dose of the vaccine strain; G2: inoculated with the parental HVT strain; G3: injected with diluent for Marek's vaccines. Each group was subsequently put in contact with SPF, day-old turkeys as follows: the first group was put in contact with eight turkeys, and each of the second and third group in contact with four turkeys. After 28 days of contact between chickens and turkeys (i.e. on D28), the chickens were euthanised while the turkeys were kept for 14 additional days (i.e. until D42). At the end of the contact period (D28) and end of the trial (D42), the viruses were tested for in the leukocytes of the birds. Anti-IBDV antibodies and anti-HVT antibodies were tested on D28 and D42.

Results:

The virus was found in both chicken inoculated groups and in the turkeys which had been in contact with them. All samples taken from control birds remained negative. Specific antibodies to the viruses tested were found on D28 in the chickens which had been inoculated with a virus and in contact turkeys on D28 and D42 while control birds remained seronegative. Chickens vaccinated either with parental or recombinant HVT do shed HVT and infect susceptible turkeys which results in viraemia and positive serology for HVT but did not result in signs of disease or lesions in turkeys infected by contact to chickens.

Study to evaluate the spread of Vaxxitek HVT + IBD vaccine in the presence of turkeys infected with HVT virus on two passages.

Methodology:

This study was carried out according to two main objectives; 1) the spread of Vaxxitek HVT + IBD in non-infected turkeys in the presence of HVT-inoculated turkeys and 2) the spread of Vaxxitek HVT + IBD in HVT inoculated turkeys.

Ten groups of day-old conventional turkeys were set up on D0 (G1, G2, G3, G4 and G5) or D28 (G6, G7, G8, G9, G10) and inoculated as follows on their day of setting-up: G1, G4: Vaxxitek HVT + IBD vaccine; G2, G6, G8: HVT virus; G3, G5, G7, G9, G10: non-inoculated. Four additional control groups were set up: G0a (D0) and G0b (D28): serological controls on the day of the group setting-up, at day-old; G11: Serology and viraemia controls (for groups set up on D0) and G12: Serology and viraemia controls (for groups set up on D28)

Contacts were performed between groups on two passages (D0 to D28 and D28 to D57 respectively) to study:

- the spread of HVT alone in non infected turkeys on one passage: G8+G9,
- the spread of Vaxxitek HVT + IBD vaccine alone in non-inoculated turkeys: G4+G5,
- the spread of Vaxxitek HVT + IBD vaccine in HVT-inoculated or potentially HVT-inoculated turkeys on two successive passages: G1+G2+G3, then G2+G6+G7,
- the spread of Vaxxitek HVT + IBD to non-infected turkeys in the presence of HVT-inoculated turkeys on two successive passages: G1+G2+G3, then G3+G5+G10.

The actual passage of the HVT and Vaxxitek HVT + IBD viruses was assessed. Virus isolation was also carried out in the birds from G11 and G12 on D28 and D57 respectively, to test the bird status with regards to HVT infection.

Results:

The viral isolation results showed that the turkeys used in the trial were not naturally infected with HVT virus. In HVT-inoculated birds, a partial serological conversion against HVT was observed from 4 weeks after inoculation ; this serological conversion was complete 7 weeks post-inoculation. In Vaxxitek HVT + IBD-inoculated turkeys, IBD antibodies were found in almost all the birds as early as 4 weeks post-inoculation. Serology results demonstrated that HVT spread from inoculated to non-inoculated turkeys. At the first passage, the spread of Vaxxitek HVT + IBD to HVT-inoculated turkeys seemed to be unlikely. The trial results also showed that there was no spread of Vaxxitek HVT + IBD to non-infected birds in the presence of HVT-infected turkeys, on two successive passages.

The results obtained confirmed the spread of HVT from turkey o turkey and that the spread of Vaxxitek HVT + IBD in turkeys was possible. They also demonstrated that Vaxxitek HVT + IBD does not spread from turkey to turkey in the context of a HVT infection.

Comparison of the vaccine infectivity in turkeys and in chickens – Dose response relationship.

Objective: this study was set up to compare the infectivity of Vaxxitek HVT + IBD vaccine in turkeys and in chickens when administered by non-parenteral route or by subcutaneous route.

Methodology: on D0, 8-day-old conventional turkeys and 6-day-old SPF chickens were inoculated with increasing doses.

The results showed that both chickens and turkeys seroconverted following inoculation of a low dose of Vaxxitek HVT + IBD by subcutaneous route and that chickens did not show seroconversion after inoculation of the vaccine by non-parenteral route whereas turkeys seroconverted partially after inoculation by non-parenteral route of a high dose of vaccine only.

Chickens were not infected by the vaccine when inoculated by non–parenteral route. These results would explain the absence of spread of the vaccine to chickens and the possible spread to turkeys. Turkeys are more sensitive to infection by non-parenteral route than chickens, but very high doses are necessary to induce a partial seroconversion.

Safety in mammalian species

Two GLP-compliant studies were provided.

Study to evaluate the non specific safety of the administration of the vaccine strain in mice in comparison with the parental non-recombinant strain.

Methodology:

Three groups of ten SPF mice were constituted: G1 inoculated with one maximal dose of the vaccine strain; G2 inoculated with the parental strain; G3 inoculated with the diluent.

Results:

No mortality occurred in any group, nor general or local reaction. Growth was identical in the three groups during the period of observation. There was no evidence of an abnormal toxicity in mouse, under the trial conditions, of the recombinant vaccine strain and of the parental HVT strain. The safety of the administration of vaccine strain at a dose near to the maximal release was demonstrated in mice.

Non-specific safety in guinea pigs of the vaccinal and of the parental strains.

Objective: to assess in guinea pigs the non specific safety of the administration of the vaccine strain in comparison with the parental non-recombinant strain.

Methodology: Three groups of four SPF guinea pigs were constituted: G1 inoculated with one maximal dose of the vaccine strain; G2 inoculated with the parental strain; G3 inoculated with the diluent.

Results: no mortality occurred in any group, nor general nor local reactions were observed. Growth was identical in the three groups during the period of observation. There was no evidence of an abnormal toxicity in guinea pigs, under the trial conditions, of the recombinant vaccine strain and of the parental HVT strain. The safety of the administration the vaccine strain at a dose near to the maximal release was demonstrated in guinea-pigs according to the European Pharmacopoeia.

OVERALL CONCLUSION ON PART III

The safety of the product has been demonstrated in several laboratory and field trials in SPF chickens and conventional birds, with respect to the maximal recommended dose and the route of administration.

The Applicant performed two studies on the safety of the administration of one dose and of an overdose, with regards to the HVT vector of the vaccine and to the recombinant nature of the vaccine. The studies were performed according to the requirements of the European Pharmacopoeia monograph for live vaccines against Marek's disease (monograph 1997:0589) and with respect to the Gumboro disease criteria (monograph 1997:0587). The safety of the repeated administration of the vaccine has been studied. No general and local reactions have been observed in these studies.

No information is available on the safety and efficacy from the concurrent use with any other vaccine, except Merial attenuated vaccines against Marek’s disease, Newcastle disease and Infectious bronchitis. It is therefore recommended that no other vaccine than these should be administered within 14 days after vaccination with the product.

The lack of spreading from chickens to chickens was suggested by the absence of serological conversion observed in sentinel chickens.. This can be explained by the very low rate of excretion of the virus and by the lack of infectivity of the vaccinal strain by non-parenteral routes.

The absence of reversion to virulence after passages in chickens has been demonstrated and the persistence of the virus has been demonstrated during at least 8 weeks. The potential of recombination of the vaccine with Marek’s disease viruses serotypes 1 and 2 has been assessed on clinical criteria and was considered as acceptable.

No residue exposure is expected.

The safety of the administration of the maximal recommended dose has been demonstrated in day-old chickens in field conditions. The vaccine is unlikely to affect male fertility. However a warning has been included in the SPC that “in the absence of data, the vaccine should not be used in breeding birds and birds in lay”.

The safety for non susceptible birds species (pheasants, ducks, partridges, quails and pigeons) and for mammalian species (mice and guinea-pigs) has been demonstrated.

No adverse effect has been observed in turkeys, which is a natural host for the virus. Shedding of the virus from vaccinated chickens and turkeys to susceptible SPF turkeys has been observed. However, the spread of the vaccine to HVT-inoculated turkeys and to non-infected birds in the presence of HVT-infected turkeys is reduced.

OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY

IV.A. INTRODUCTION

The vaccine is intended to broiler chickens as well as to future egg-layers or future breeders. Like other Marek's vaccines, it has to be inoculated as early as possible (at day-old). At this moment, passive immunity against IBD is very high and, therefore, vaccine against IBDV must be insensitive to maternally derived antibodies (MDA). This is the aim of this recombinant vaccine.

Marek's disease (MD) is the most common lymphoproliferative disease of chickens. MD is prevented by vaccination of day-old chicks with monovalent or bivalent live virus vaccines of various type. Amongst these, HVT strains (serotype 3) are commonly used to vaccinate day-old birds. HVT may be prepared in a cell-free form as a freeze-dried (lyophilised) vaccine or in a cell-associated ("wet") form. The parental strain of Vaxxitek HVT + IBD (FC-126 strain) is a naturally avirulent HVT which is widely and classically used in vaccination against Marek's disease, either as a monovalent vaccine or in combination with serotype 1 and 2 strains in bivalent vaccines against the very virulent strains of MDV. Vaccination reduces clinical disease, but not persistent infection by MDV. The vaccine viruses are also carried throughout the life of the chicken.

No interference between the Marek's disease serotype 1 Rispens vaccine and Vaxxitek HVT + IBD regarding the efficacy against IBDV was shown.

Infectious Bursal Disease (IBD) is widely spread world-wide, and particularly where avian industry is intensive. For many years, Gumboro disease has constituted a serious problem for the poultry industry, and the recent 're-emergence' of the Infectious Bursal Disease Virus (IBDV) in the form of antigenic variants or hypervirulent strains has taken a very heavy toll on the sector. Direct losses are linked to specific mortality, and depend on the dose and virulence of the inoculum, the age and breed of the animals, and the presence or absence of passive immunity. The disease's indirect economic impact is also quite considerable due to virus-induced immunosuppression and/or potential interactions between IBDV and other viruses, bacteria, or parasites. These indirect losses take the form of secondary infections, growth retardation and condemnation of carcasses at the slaughterhouses. Moreover, the increased use of antibiotics against secondary infections constitutes a growing public health concern.

The most recent survey of international poultry specialists, conducted by World Poultry, highlighted continuing concern in the sector over the sanitary status of poultry. Gumboro disease topped the list of the most serious poultry diseases.

Classical serotype 1 IBDV vaccines induce a good protection but the actual problem for control of the disease has become the interference of maternally derived antibody in the establishment of the vaccination schedule. The development of safe vaccines that could either transmit a high passive immunity that could protect broilers during the whole growing period or prime an immune response before or at hatching in the presence of passive immunity has become indispensable. In this context, as they are safe and insensitive to passive neutralisation, recombinant vaccines might have an advantage over other approaches.

Although the role of cell-mediated immunity cannot be ruled out, protection against IBDV is known to be correlated with the levels of neutralising antibodies. Neutralising epitopes are located on the VP2 structural protein of IBDV. This protein is expressed in the recombinant Vaxxitek HVT + IBD vaccine.

Chickens develop immunological responsiveness well before hatching, and early protection against infections, such as Marek's disease has been demonstrated.
In order to demonstrate the efficacy of the Vaxxitek HVT + IBD vaccine by *in ovo* route, tests were carried out both in the laboratory and in the field.

Trials were carried out in compliance with provisions led down in the requirements in force at the time of implementation of trials:

- The general requirements, concerning live vaccines, of Directive 92/18/EEC (now 2001/82/EC) Part 8, have been fulfilled;
- The specific guidelines for the production and control of avian live and inactivated viral and bacterial vaccines;
- The European Pharmacopoeia monographs were used as specific guides (*Vaccinum bursitidis infectivae aviariae cryodesiccatum*, No.0587; *Vaccinum morbi marek vivum*, No.0589).

The vaccine containing a live recombinant virus, the described laboratory trials were concerned by the Directive 90/219/EEC. Appropriate authorisation was obtained.
Besides, clinical trials involved a deliberate release into the environment under the terms of Directive 90/220/EEC (now repealed and replaced by 2001/18/EC). The appropriate authorisation was sought from France prior to the trials. The appropriate authorisation was provided.

IV.B. GENERAL REQUIREMENTS

The Applicant has performed laboratory and field trials to support the claims of the product. Eight of these studies were conducted to support the efficacy against Gumboro disease while only one was related to Marek's Disease (potency test). Further studies to support the claim against Marek's disease were also presented. All studies have been performed using the minimal guaranteed dose of the product (3.0 log10 PFU) administered in the target species.

Trials were carried out in compliance with provisions led down in the requirements in force at the time of implementation of trials (including the European Pharmacopoeia and the OIE Manual of Standard for Diagnostic Tests and Vaccines).

Data from laboratory and field trials

- The efficacy by subcutaneous route was demonstrated and approved with a recommended minimum dose of 3.0 log10 PFU. The *in ovo* route requires a higher minimum dose (3.6 lof10 PFU) for appropriate efficacy with regards to the two diseases. The minimum guaranteed dose will be finally 3.6 log10 PFU for the two routes.
- As required in Directive 2001/82/EC, the *in ovo* efficacy of Vaxxitek HVT + IBD vaccine has been demonstrated:
  - in the categories of the target species recommended for vaccination (embryonated eggs aged 18 days of incubation);
  - by the recommended route of administration (*in ovo* route);
  - using the proposed schedule of administration (single injection of 50 µl);
  - using the minimum dose (3.6 log10 PFU per dose)
- The influence of maternally derived antibodies on the efficacy of vaccine was evaluated.
- Data from trials support the claims.

The vaccines batches used for efficacy trials were produced according to the manufacturing process described in the quality part of the registration dossier except for one trial. The equivalence of processes on efficacy was demonstrated.

A glossary is provided for clarification.

GLOSSARY

HVT	Herpesvirus of turkeys	MSV	Master Seed Virus (vHVT013-69)
IBD	Infectious Bursal (Gumboro) Disease		
IBDV	Infectious Bursal Disease Virus		
VvIBDV	Very virulent IBDV		
MD	Marek's Disease	HVT FC-126	Parental non-recombinant strain
MDV	Marek's Disease Virus	SPF	Specified Pathogen Free
MDA	Maternally derived antibodies	EP	European Pharmacopoeia
PFU	Plaque Forming Unit	BF	Bursa of Fabricius

IV.C. DOSE RESPONSE RELATIONSHIP/VALIDATION OF MINIMUM TITRES

1. Potency tests / Efficacy against challenge

According to the claim of the vaccine, the potency of the vaccine was studied with respect to Gumboro and Marek's diseases.

1.1 Efficacy with respect to IBD:

1.1.1 Dose titration against a classical strain of IBDV

Objective: to evaluate the dose-response relationship of Vaxxitek HVT + IBD against the infectious bursal disease (IBD) in SPF chickens.

Methodology:

Five groups of twenty SPF day-old chicks were constituted on day 0 (D0) of trial and were treated with various doses (G1, G2, G3 and G4). Group G5 was not vaccinated. All groups were challenged on D14.

The challenge was performed according to the EP monograph requirements. The challenge strain was the virulent Faragher strain of IBD virus. All birds were clinically monitored for 10 days after challenge. Dead animals were post-mortem examined. On D24, surviving animals were euthanised and post-mortem examined, and the bursae of Fabricius were collected for histological examinations. Ten chicks (G6), neither vaccinated nor challenged, of the same age and origin as the other birds, were euthanised and sampled in the same manner. They were used as controls for histological examinations. Protection percentages were calculated according to requirements of European Pharmacopoeia (EP) monograph relating to live IBD vaccines (1997:0587).

Protection (total absence of mortality and of bursal lesions) was conferred by the vaccine for groups G3 and G4. The predictive PD50 and PD90 values of Vaxxitek HVT + IBD vaccine against classical IBDV in SPF day-old chicks vaccinated s.c. were defined.

1.1.2 Efficacy against a very virulent strain of Gumboro disease virus (vvIBDV)

Objective: to study the potency of Vaxxitek HVT + IBD vaccine inoculated by subcutaneous route (s.c.), against a very virulent strain of Gumboro disease virus (vvIBDV) in SPF chicks, according to the EP requirements (1997: 0587).

Methodology:

Two groups (G2, G3) of 50 SPF embryonated hen eggs aged 18 days were constituted on day-4 (D-4) of trial. At hatching, some chicks (subgroups G2a and G3a) were challenged on D14, as required by

EP monograph (1997: 0587). Group G2a were inoculated in ovo with Marek sterile diluent on D-4; G3a- inoculated at day-old with 3.0 log10 PFU of Vaxxitek HVT + IBD vaccine (S.C. route). All G2a and G3a birds were challenged on D14 with a very virulent IBDV strain given by ocular route.

Clinical observations were carried out daily for 10 days after challenge. Animals that died before the end of observation period (D24) were necropsied for signs of Gumboro disease. On D24, all the surviving animals were euthanised and necropsied. Particular attention was paid to the macroscopic appearance of the bursa of Fabricius. Histological observations of bursae of Fabricius of all animals necropsied on D24 were carried out. Lesions were scored in accordance with the EP monograph (1997:0587).

Results

Subcutaneous vaccination at one day of age with the minimum guaranteed dose of RMB533 vaccine protected birds against challenge with vvIBDV on D14. The potency of the vaccine was in accordance with the EP monograph.

Vaxxitek HVT + IBD – vHVT013-69 Recombinant vaccine – Dose titration study after mechanical in ovo vaccination in SPF chickens.

Objective: to assess the dose response effect for the vaccine, when inoculated *in ovo*.

Methodology: six groups of 25 embryonated eggs aged 18 days were set up on D-3 and injected as follows:

- G0: unvaccinated controls, and challenged on D14;
- G1 to G5: inoculated *in ovo* with various doses of Vaxxitek HVT + IBD, and challenged on D14 with a very virulent strain of IBDV;

- A clinical examination was carried out daily, and sick and dead animals were recorded.
- All the chickens which died before the end of the 10-day observation period following the challenge were necropsied for signs of Gumboro disease (lesions of the bursa of Fabricius, muscular hemorrhages).
- At the end of the trial (D24), all surviving animals were euthanased and necropsied for signs of Gumboro disease.
- The bursae of Fabricius were sampled for histology. Lesions were scored in accordance with the European Pharmacopoeia (monograph 1997: 0587)

Results:

After challenge, the mortality rate in controls was 45%, and all the surviving animals in this group showed severe histological lesions of the bursa of Fabricius. These results validate the challenge with regards to the European Pharmacopoeia potency test for live vaccines against Gumboro disease.

The Ph. Eur. protection threshold (90%) can be considered as superseding the Ph. Eur. requirements when 3.6 log10 are administered.
The analysis of the results showed a relation between the *in ovo* inoculated dose and the protection

The challenge strain used was strongly heterologous and suitable for demonstration of efficacy: the study conditions are more severe and mimic a wild field infection.

1.2 Efficacy with respect to Marek's disease

Objective: to assess the dose response effect for Vaxxitek HVT + IBD vaccine against Marek's disease (MD), when subcutaneously (s.c.) administered to day-old SPF chickens.

Methodology:

Groups of chicks aged one day each were set up on D0 and inoculated s.c. (in 0.2 ml) with the Vaxxitek HVT + IBD vaccine at various doses G1, G2, G3 and G4). Group G5 was unvaccinated and acted as positive challenged control group and G6 was unvaccinated and unchallenged.

At 5 days old (D4), all the vaccinates (G1 to G4) and G5 were each intraperitoneally challenged with GA22 Marek's disease virus. The birds were observed for 70 days post-challenge, then euthanised on D74 and necropsied for signs of Marek's disease according to the requirements of the EP monograph for live vaccines against Marek's disease (1997:0589). Any bird dying prior to the end of the trial was necropsied to confirm infection with MD virus.

The minimum protective dose of Vaxxitek HVT + IBD against MD administered to SPF chickens at one day of age was established. This dose provided more than 80% protection against a virulent Marek's disease challenge, as required by the E.P.

Potency of the vaccine against Marek's disease GA22 challenge in day-old SPF chickens (SC route)".

Objectives: the aim of this trial was to assess the potency of Vaxxitek HVT + IBD when subcutaneously (SC) administered to day-old SPF chickens, against a Marek's disease (MD) challenge with GA22 strain.

Methodology: two groups named GA and GB containing 38 chicks each were set up on D0, and each day-old chick from GA was SQ inoculated with 3.0 log10 PFU of vaccine. The other chicks (GB) remained non-vaccinated and served as challenged controls. On D9, 30 chickens in each GA and GB were each intraperitoneally challenged with GA22 Marek's disease virus. The challenged birds were observed for 70 days post-challenge (D9 to D79), then euthanised on D79 and post-mortemed for signs of Marek's disease (hypertrophy and/or tumour formations on main organs and /or on nerves).

Results

The relative protection score in the RMB 533 vaccinates (GA) was 91.3%. This result is markedly higher than the minimum rate (80%) required in the European Pharmacopoeia. This trial shows that the minimal dose of the vaccine reduces mortality, clinical signs and lesions of the Marek Disease against a challenge performed 9 days after vaccination, in accordance with EP 1997:0589.

Vaxxitek HVT + IBD – Potency - Minimum protective dose against Marek's disease of the vaccine administered in ovo to 18-day-old SPF chicken embryonated eggs.)

Objective: to assess the dose-response effect and the minimum protective dose of Vaxxitek HVT + IBD against Marek's disease (MD), when administered *in ovo* to 18-day-old SPF chicken embryos.

Methodology: one hundred and sixty (160) SPF eggs at 18 days of embryonation were divided into 4 groups of 40 named G1 to G4 at day 0 (D0). Each group was inoculated as follows:

- G1 to G4 inoculated *in ovo* with 0.05 ml of Vaxxitek HVT + IBD vaccine containing various amount of virus
- At 5 days old (D7), all G1 to G4 and control birds were intraperitoneally challenged virulent Marek's disease virus.
- The birds were observed for 70 days post-challenge (D7 to D77), then euthanased on D77 and post-mortemed for signs of Marek's disease (hypertrophy and/or tumour formations on main organs and /or on nerves). Any bird dying prior to the end of the trial was necropsied to confirm infection with MD virus.

The challenge caused clinical and lesions due to MD in 96.7% of the challenged controls. This result is above the minimum rate (70%) stated in the European Pharmacopoeia monograph for live vaccines against Marek's disease (1997:0589). It thus validated the trial, showing the virulence of the challenge strain and the susceptibility of the birds used.

Under the conditions of the trial, the minimum protective dose against Marek's disease of Vaxxitek HVT + IBD administered *in ovo* was defined, according to the European Pharmacopoeia criteria.

The recommended dose of 3.6 log10 PFU is greater than the minimum vaccine field dose of each type of Marek's vaccine in the OIE Manual of Standards for Diagnostic Tests and Vaccines and accepted as the commercial threshold value for immunisation.

As a minimum efficacious doses were shown to protect respectively against to Gumboro and Marek's diseases, in compliance with the corresponding Ph. Eur. monographs, the efficacy of Vaxxitek HVT + IBD vaccine is demonstrated using a 3.6 log10 PFU minimum dose. Challenge models with respect to Gumboro disease and Marek's disease have been performed according to the corresponding EP monographs.

The results of the two above studies are fully compatible with those obtained with the subcutaneous administration of the vaccine to day-old chicks with regard to Gumboro disease and Marek's disease. The onsets of protection are in accordance with those already demonstrated for the subcutaneous route at day old.

2. Duration of Immunity

2.1 Persistence of vaccine strain in vaccinated birds

Objective: to evaluate the persistence of vaccine strain and the kinetics of Gumboro antibody titres in vaccinated SPF chicks.

Methodology:

Four groups of SPF day-old chicks were constituted: G0, unvaccinated (used for serological control); G1, injected s.c. on D0 with one dose (3.0 log10 PFU) of Vaxxitek HVT + IBD vaccine; G2, injected s.c. on D0 with one dose (3.0 log10 PFU) of the parental HVT strain; G3, unvaccinated controls. During the trial, presence of virus in G1, G2, and G3 animals was assessed by virus re-isolation tests. Serological monitoring was also carried out by titration of anti-IBDV seroneutralizing antibodies.

Persistence of HVT virus in SPF chicks was demonstrated for at least 8 weeks, for the parental and recombinant virus, as well. A lower virus load was evidenced in birds vaccinated with Vaxxitek HVT + IBD than in birds inoculated with HVT parental strain. In addition, a steady increase in serological titres against Gumboro disease was observed up to 61 days after vaccination with Vaxxitek HVT + IBD.

2.2 Duration of protection

Objective: to study the duration of immunity conferred by Vaxxitek HVT + IBD vaccine inoculated by subcutaneous route (s.c.), against a very virulent strain of Gumboro disease virus (vvIBDV) in SPF chicks.

Methodology:

Two groups (G2, G3) of 50 SPF embryonated hen eggs aged 18 days were constituted. Subgroups G2b and G3b were constituted at hatching and were challenged on D56 for assessment of duration of immunity. G2b, inoculated in ovo with Marek sterile diluent on D-4; G3b, inoculated at day-old (D0) with 3.0 log10 PFU of Vaxxitek HVT + IBD vaccine (S.C. route). All G2b and G3b birds were challenged on D56 with a vvIBDV strain. Clinical observations were carried out daily for 10 days after challenge.

Histological observations of bursae of Fabricius were carried out. Serological response of birds (anti-IBDV antibody titres) was investigated over the study period.

Results:

No dead or sick animal and no macroscopic or histological lesions of the BF were observed in any G3b vaccinates. Thus, protection rate was 100% in all respects. Serological results showed a steady increase of the antibody titres in vaccinated animals between D14 and D56. Subcutaneous vaccination at one day of age with the minimum guaranteed titre of Vaxxitek HVT + IBD protected birds against challenge on D56. The duration of immunity was at least 8 weeks.

3. The influence of maternal antibody on the efficacy of the vaccine

3.1 Efficacy with respect to Gumboro disease

3.1.1 Influence on serological response

Objective: to evaluate the potency of Vaxxitek HVT + IBD vaccine in chickens with maternally-derived antibodies.

Methodology:

Three groups of 14 conventional day-old chicks were constituted on day 0 (D0) of trial: G1, unvaccinated control group; G2, vaccinated s.c. with 3.0 log10 PFU of Vaxxitek HVT + IBD vaccine; G3, vaccinated with a usual live vaccine against infectious bursal disease (intermediate strain) by ocular route. A fourth group (G0) of 10 conventional day-old chicks of the same origin as other groups was included for serological investigation on D0. Blood samples were taken in order to titrate anti-IBDV antibodies.

Results: anti-HVT and anti-IBDV antibodies were detected on D0 in group G0. Subsequently, anti-IBDV antibodies measured with both methods decreased steadily in controls (G1) and in birds vaccinated with the usual vaccine (G3). Conversely, the decrease in the two types of anti-IBDV antibodies stopped after D21 in Vaxxitek HVT + IBD-vaccinated birds (G2). Serological titres were very significantly (p=0.000) higher on D42 in G2 than in the other two groups.

The trial demonstrated that maternally-derived antibodies had no effect on serological response to Vaxxitek HVT + IBD vaccine.

3.1.2 Influence on protection against challenge

Objective: to study the potency of Vaxxitek HVT + IBD vaccine inoculated by subcutaneous route (s.c.), against a very virulent strain of Gumboro disease virus (vvIBDV) in conventional broilers.

Methodology:

A total of 110 conventional day-old chicks were randomly allocated to the following three groups: G0, 10 unvaccinated controls used for serological control on D0; G1, 51 vaccinates s.c. on D0 with 3.0 log10 PFU of Vaxxitek HVT + IBD; G2: 49 animals inoculated s.c. on D0 with sterile diluent. On D21, G1 and G2 chicks were challenged. These subgroups were named G1a and G2a, respectively. Other G1 and G2 birds were challenged on D42. These subgroups were named G1b and G2b, respectively. Both challenges were carried out with the vvIBDV strain 91-168.

Birds were clinically observed daily for 10 days post-challenge. Particular attention was paid to the signs of Gumboro disease (lesions of the bursa of Fabricius, muscle haemorrhages). The bursae were sampled for histology.

Results: The high antibody titres measured on D0 confirmed that the trial was carried out in the presence of maternal antibodies. No vaccinated animal showed any gross lesions of its bursa or severe histological lesions. On the contrary, most controls had typical gross lesions of the bursa of Fabricius, and some controls showed severe lesions of the bursa. After the D42 challenge some animals died in the controls (G2b). All the controls surviving on D52 showed typical gross lesions of the bursa. Some had severe histological lesions. On the contrary, all the vaccinated birds (G2a) were protected from the clinical, macroscopical and histological perspective.

Protection against vvIBD has been demonstrated in conventional chicks vaccinated s.c. at one day of age with a low dose of Vaxxitek HVT + IBD, in the presence of maternally derived antibodies against a challenge performed using a very virulent strain of IBD 3 and 6 weeks after vaccination.

The trials presented in section 2. "Duration of immunity" and in section D. "Field trials" were performed using conventional embryonated eggs, showing high levels of maternally derived antibodies at hatching.

Two trials fully demonstrate the absence of influence of MDA on the vaccine efficacy against infectious bursal disease.
One trial fully demonstrates the absence of influence of MDA on the vaccine efficacy against Marek's disease.

Consequently, the long experience of vaccination against Marek's disease using HVT strains, the current scientific knowledge of immune response against Marek's disease viruses, and the data provided were sufficient to demonstrate the efficacy of Vaxxitek HVT + IBD in the presence of maternal derived antibodies.

3.2 Efficacy with respect to marek's disease

Study 1. Objective: the purpose of this study was to determine the minimum protective dose of the vaccine, administered subcutaneously to chickens in presence of MDA, at day of age and challenged at five days of age with GA22 Marek's disease challenge virus.

Methodology: the chicks were divided into groups of 35 and each chick was s.c. inoculated with various doses of the vaccine. Thirty (30) hatchmates remained non-vaccinated and served as challenged controls. Four days post-inoculation, the vaccinates and 30 controls were each challenged with GA22 Marek's disease virus. The birds were observed for 49 days post-challenge, terminated, and examined for gross clinical signs of Marek's disease. They were euthanased and post-mortemed for signs of Marek's disease.

Results: Protection against challenge was shown in the dosed birds. Under the conditions of this test, the minimum protective dose of vaccine, administered s.c. to commercial broiler chickens at day of age against a virulent Marek's disease challenge was established.

Study 2. Objective: the study was carried out to assess the potency of Vaxxitek HVT + IBD vaccine against Marek's disease when it was administered to newly-hatched conventional chickens under field conditions.

Methodology: 2 groups of approximately 300 birds were set up and vaccinated on D0 and reared under commercial conditions. For each of the challenges, a group of 30 young SPF chickens was included to validate the Marek's disease challenge. The vaccine was administered subcutaneously to the pullets when they were 1-day-old. The dose used was low.

The potency was assessed serologically and by 2 experimental challenges; the 1st challenge was carried out 9 days after vaccination and a 2nd challenge was carried out 127 days after vaccination in an attempt to show a duration of immunity following the vaccination (although a poor sensitivity to challenge was expected at this age). For each challenge, a sub-group of 30 birds was selected at

random from each of the rearing groups G1 and G2. These birds were challenged with a virulent strain of Marek's disease virus .

Results: the serology confirms that vaccination on D0 was carried out in the presence of maternal antibodies and clearly demonstrates seroconversion in the vaccinates. Following the 1st challenge, the Relative Protection Score (RPS) for the G1 birds compared to the G2 birds was 56%. Following the 2nd challenge, only a few birds in each of groups G1 and G2 showed signs of Marek's disease. This was expected to be due to the poor susceptibility of these older chickens to infection with Marek's disease virus; Marek's disease has a long incubation period and it is accepted that outbreaks that occur in older birds are due to early infection.

The study demonstrated some effectiveness of the vaccine in conventional commercial laying chickens under field conditions, against a virulent Marek's disease challenge carried out 9 days after vaccination of the birds.

Vaxxitek HVT + IBD – vHVT013-69 recombinant vaccine – Potency of the vaccine against a very virulent strain of Gumboro disease virus (vvIBDV) after in ovo vaccination in conventional broilers. Duration of immunity).

Objective: to assess the duration of immunity of Vaxxitek HVT + IBD against a very virulent Gumboro disease virus (vvIBDV) in conventional broilers, when administrated by the in ovo route.

Methodology:

- Four groups of embryonated eggs aged 18 days were set-on Day -3 (D-3) and inoculated as follows:
  - G3 unvaccinated controls, (n=10 and n=40 respectively);
  - G1 vaccinated with minimum dose of Vaxxitek HVT + IBD, (n=40);
  - G2 vaccinated with above minimum dose of Vaxxitek HVT + IBD, (n=40).
  - On D21, 20 animals of each group 1 to 3 were randomly and challenged with a vvIBDV on D21 or on D49.

- A clinical observation was carried out daily for 10 days post-challenge. Any chicken that died after challenge was necropsied for signs of Gumboro disease. At the end of the observation periods (D31 and D59), all the surviving chickens were euthanased and necropsied. Particular attention was paid to the signs of Gumboro disease (lesions of the bursa of Fabricius, muscle haemorrhages). The bursae were sampled.
- Anti-IBDV antibodies were looked for in the sera of randomly chosen birds in each group sampled on each date of challenge (D21 and D49).

Results:

- Most of the controls showed clear specific gross lesions of the bursa after the challenge. The proportion of controls showing clinical signs and/or intermediate or severe histological lesions of the bursa was 94.7% and 100.0% after the challenge carried out at 21 and 49 days of age respectively.
- There was 65.0% and 70.0% protection after the first challenge in the groups vaccinated with minimum and above minimum dose respectively.
- After the second challenge, there was 93.3% and 71.4% of protection respectively. The difference between the number of protected chickens in the control and vaccinated groups is highly significant.

Serology:

- All the sera tested on D0 in the serological control group (G0) were positive.

- A decrease in the mean antibody titres in all groups was observed between D0 and D21. From D21, the decrease went on in controls. On D49, a serological conversion was observed in the vaccinated groups.

In the field study the applicant performed a challenge at 3 and 6 weeks of age in chickens vaccinated *in ovo* in presence of high m.d.a. and showed protection against a very virulent strain.

The role of seroneutralising antibodies in protective immunity was shown. A direct correlation between antibody level and protection against IBD has thus been demonstrated, and the data were sufficient to grant a 9-week duration of immunity by S.C. route
During the vaccine development for *in ovo* administration, it was found that the minimum vaccine dose should be increased to obtain similar protective antibody levels.

The serological response after vaccination using S.C. route or *in ovo* route are similar. In laboratory and field conditions, antibody kinetics are exactly the same, from the vaccination until the end of the studies.

The chickens used for the duration of immunity after *in ovo* vaccination were challenged at 2 weeks and 7 weeks of age A satisfactory protection level against challenge was demonstrated at each date, in accordance with the proposed indication. The birds showed high maternal antibody titres at hatching, and after vaccination satisfactory antibody titre at two weeks of age, and at seven weeks of age.

As a conclusion, 9 weeks duration of immunity after S.C. administration was approved by the CVMP.

Taking into account that the minimum dose has been increased from 3.0 log10 for the S.C. route to 3.6 log10 PFU for the *in ovo* route in order to maintain similar level of efficacy for the 2 routes of administration, the same analysis regarding duration of immunity was conducted for the *in ovo* route.

These compiled results showed that the antibody levels obtained with both routes were equivalent, correlated to the protection level against challenge, and in line with antibody kinetics observed in SPF birds.
As for laboratory trials, the antibody kinetics and protection levels obtained in the field with both routes were very similar.

The duration of protection of 9 weeks was accepted for the subcutaneous route following vaccination of day-old chicks by. Challenges against very virulent IBDV were performed up to 8 weeks of age and correlation between anti-IBDV seroneutralising antibodies and protection against challenge was clearly demonstrated.
A field trial conducted in field conditions showed birds with very satisfactory IBDV SN titres after 9 weeks of.
The results of serological responses obtained using the subcutaneous route and the *in ovo* route are superposable.. A duration of protection of 9 weeks against Infectious Bursal disease is accepted for the *in ovo* route.

The duration of immunity regarding Marek's disease has been evaluated in Section D. "Field trials".

4. Compatibility/interference studies

Objective: to assess a potential adverse effect of the Marek's disease serotype 1 Rispens vaccine (CRYOMAREX Rispens) on the Vaxxitek HVT + IBD vaccination, by serological follow up.

A serological follow-up when the vaccine is associated with a live Marek's disease vaccine showed a delay in serological conversion of anti-IBDV antibody titres. Additional data demonstrating the absence of interference between Vaxxitek HVT + IBD and a serotype 1 live Marek vaccine in laying chickens were presented.

Objective: the aim of this trial was to assess the safety of Vaxxitek HVT + IBD vaccine and its interaction with Marek's disease serotype 1 Rispens vaccine in laying chickens. This study was based on the monitoring of the laying performances of SPF pullets vaccinated at day-old.

The safety of Vaxxitek HVT + IBD combined with Marek's disease serotype 1 Rispens vaccine in layer chickens was demonstrated. No interaction between the 2 vaccines on laying performances and on viability during the lay was observed. The compatibility with routine broilers vaccination programmes against Infectious bronchitis and Newcastle disease was assessed. The adequacy of Vaxxitek HVT + IBD use in a complete vaccination programme for future layers and breeders was demonstrated in two trials. The efficacy of the vaccination scheme proposed in the SPC using Vaxxitek HVT + IBD vaccine against IBD and Marek's disease (i.e. one injection at day-old) is justified, serologically and by challenge, for the category of chickens indicated.

IV.D. FIELD TRIALS

First trial

Objective: to assess by serological monitoring the potency against Gumboro disease (IBD) of Vaxxitek HVT + IBD vaccine administered by subcutaneous route (s.c.) in day-old chickens in field conditions.

Methodology:

A total number of about 60,700 conventional day-old chickens distributed in two farms was included in the trial. In each farm, day-old chicks were allocated to two groups: G1 chicks were subcutaneously vaccinated at day-old with 3.0 log10 PFU of Vaxxitek HVT + IBD; G2 chicks served as controls, and were administered a reference live vaccine against Gumboro disease commonly used in the field, according to a standard vaccination scheme (2 successive doses). The birds were clinically monitored throughout the trial. At slaughter (about 6 weeks of age), the number of birds slaughtered, the mean carcass weight and the percentage condemnation were noted per group, for each farm. The feed conversion indexes were calculated, taking into account the feed consumed. Serological monitoring of the chicks from each group was implemented in the two farms. Individual sera were tested for IBD antibodies.

Results:

Zootechnical data were equivalent in G1 (Vaxxitek HVT + IBD) and G2 (controls), for the two farms. In both farms, an early mortality syndrome was suspected, which induced higher mortality than expected within the first weeks of age. This mortality was around 10%, which was acceptable. The results were the same in Vaxxitek HVT + IBD vaccinates and in controls in the two farms. The technico-economical results were satisfactory in both farms, in Vaxxitek HVT + IBD vaccinates (G1) and in the controls (G2). There was no marked difference between the Vaxxitek HVT + IBD vaccinates and the Reference IBD vaccinates from the zootechnical viewpoint.

High levels of IBD antibodies were found in day-old chicks. A decrease in the antibody titres was noted during the first two weeks of the trial, then a clear serological conversion was observed in all the flocks from D28. In G1 groups (Vaxxitek HVT + IBD vaccinates), the antibody levels remained indeed stable from this date in farm n° 2, while they had a clear increase in farm n° 1 demonstrating thereby an active humoral immunization. The same serological profile was seen in the controls vaccinated with the reference IBD vaccine (G2).

The potency of Vaxxitek HVT + IBD vaccine after subcutaneous vaccination of conventional day-old chickens in field conditions has been demonstrated serologically.

Second trial

Objective: this trial aimed at studying the potency of Vaxxitek HVT + IBD vaccine used in field conditions.

It included about 36,000 conventional day-old chickens distributed in two buildings (two successive broiler flocks). The potency was assessed serologically and by experimental challenges with a very virulent strain of Gumboro disease virus (vvIBDV).

Methodology: Birds of each broiler house were split into four groups: G1, birds vaccinated with Vaxxitek HVT + IBD at day-old, reared on the farm site; G2, birds vaccinated with the reference IBD vaccine (intermediate strain) at 2 weeks of age (2 successive doses), G3, birds vaccinated with Vaxxitek HVT + IBD at day-old in the field, transferred site to confined animal facilities; G4, unvaccinated control hatchmates, transferred at day-old to confined animal facilities.

Vaxxitek HVT + IBD vaccination was carried out in the farm by subcutaneous injection of 3.0 log10 PFU per bird, using an automated vaccinator. The date of Vaxxitek HVT + IBD vaccination was considered as D0 of the trial. The reference live vaccine against Gumboro disease was used according to a standard vaccination scheme (2 successive doses 4 days apart).

Two challenges were carried out with a vvIBDV strain in groups G3 and G4: an early challenge on D20 and a late challenge on D37. Unchallenged negative controls (n = 10) were kept for each challenged group. Subgroups of animals from groups G1 and G2 were transferred a few days before challenge. The remaining birds in the farm site (G1 and G2) were reared in accordance with the methods usually carried out in the farm. They were monitored from the zootechnical viewpoint. A serological follow-up was also carried out in the four groups (test for IBD antibodies).

Results:

The zootechnical data were equivalent in Vaxxitek HVT + IBD vaccinates and in the controls, the technico-economic results at slaughter were also fully satisfactory. None of the data recorded suggested a wild IBDV passage in the farm during the trial. The same serological profile was observed in the vaccinates (G1, G2, G3) with a decrease in the antibody levels within the first weeks followed by the increase or stabilisation of IBD titres by serological conversion after D20.

After the early challenge (D20), the results obtained demonstrated the onset of the immunity conferred by Vaxxitek HVT + IBD, with a statistically significant protection against vvIBDV as compared with the unvaccinated controls, while a reference IBD vaccine afforded no protection yet against challenge at this date, taking into account the low interval between vaccination and challenge.

After the late challenge (D37), there was a highly significant difference between the number of protected chickens in the Vaxxitek HVT + IBD vaccinates and unvaccinated controls.

The number of protected birds in the group administered a reference IBD vaccine (G2) was also significantly higher than in the unvaccinated controls. The protection obtained in the Vaxxitek HVT + IBD vaccinates was significantly higher than in the other IBD vaccinates (G2).

The efficacy and the duration of immunity of Vaxxitek HVT + IBD vaccine after subcutaneous vaccination of conventional day-old chickens in field conditions has been demonstrated against a vvIBDV challenge. Vaxxitek HVT + IBD vaccine also demonstrated its efficacy in comparison with a standard vaccination protocol (including 2 administrations versus a single one). Immunity has been shown to persist at least as long as the economic life span of broilers.

In support of these data the influence of maternally derived antibodies on HVT seroconversion was studied in a **further trial** performed in field. The vaccine was administered subcutaneously to the pullets when they were 1-day-old. The dose used was a low dose of 3.0 log10 PFU per bird administered in 0.2 ml. The serology confirms that vaccination on D0 was carried out in the presence of maternal antibodies and clearly demonstrates seroconversion in the vaccinates.

A further trial conducted in field conditions showed birds with very satisfactory IBD titres. Low titres at the end of the trial do not mean that vaccine take was not satisfactory. All trials conducted with conventional birds showed a decrease in anti-IBD antibody levels (lowest titres around 4-5 weeks of age) then an increase demonstrated as early as 6 weeks of age. Antibody levels increased regularly or at least remained stable in all studies of longer duration.

The birds included in this trial showed high levels of maternally derived antibodies. Absence of mortality following an IBDV infection is not uncommon in the field, and the challenge results were expected in this category of birds. A classical IBD challenge strain such as Faragher does not induce either mortality nor lesions in conventional animals. That is the reason why a vvIBDV strain was used, to induce severe lesions of the Bursa of Fabricius. Although mortality was followed to be exhaustive, the efficacy of the vaccine with regards to mortality is difficult to demonstrate in these conditions. However, other trials presented in the dossier demonstrated that, when mortality occurred, the vaccinated birds have been protected.

Vaxxitek HVT + IBD vaccine – Recombinant live vaccine vHVT013-69 - Field trial – Potency and duration of immunity against Gumboro disease after in ovo vaccination, assessed by serology and vvIBDV challenges.)

Objective: to study the potency of Vaxxitek HVT + IBD vaccine administered *in ovo* in field conditions.

Methodology:

- More than 100,000 eggs were included in the study, divided into three flocks (F1, F2, F3) which were successively bred in different conventional broiler Farms.
- The potency was assessed serologically and by experimental challenges with a very virulent strain of Gumboro disease virus (vvIBDV) carried out in birds transferred to MERIAL's confined animal facilities.
- For each flock, two groups were set up:
  - G1: Broilers vaccinated with Vaxxitek HVT + IBD *in ovo*.
  - G2: Control broilers injected *in ovo* with sterile diluent, and vaccinated with the reference IBD vaccine by 2 weeks of age on the field.

- The *in ovo* injections were mechanically carried out at the hatchery in eggs of at least 18 days of embryonation. The day of hatch was considered as Day 0 (D0) of the trial for each flock.
- two challenges were carried out with a vvIBDV strain in Merial's animal facilities: an early challenge by 3 weeks old (D21 or D22) and a late challenge by 6 weeks old (D40 or D42). There were at least 20 birds challenged per date and per group. Ten unchallenged negative controls were kept for each challenged group. At each challenge, the birds were considered as positive if they died and/or if they had severe histological lesions of their bursa of Fabricius 10 days post-challenge.

Results:

- The zootechnical data were equivalent in Vaxxitek HVT + IBD vaccinates and in the controls, the technico-economic results at slaughter being also fully satisfactory.

Serology results confirmed that the broilers used had high maternally–derived IBD antibody titres at hatching. The antibody levels decreased afterwards until approximately 3 weeks of age, due to the decay of maternal antibodies. The IBD antibody titres went on decreasing afterwards in the non-vaccinated G2 birds maintained in Merial's facilities until the last blood sampling by 6 weeks of age. In the Vaxxitek HVT + IBD vaccinates (field + confined facilities), an increase or stabilisation of the IBD titres was observed due to serological conversion as from D20 to

- The vaccine take in G2 with the standard IBD vaccine seemed to be unsatisfactory in the first two flocks, but a clear seroconversion was seen in F3. The antibody levels obtained with the standard vaccine in G2 birds were yet lower than those observed in the Vaxxitek HVT + IBD vaccinates.

- Challenge

  After the early challenge (D21/22), the results obtained allowed to evidence the onset of the immunity conferred by Vaxxitek HVT + IBD, with a statistically significant protection against vvIBDV as compared with the unvaccinated controls.

  After the late challenge (D40/42), there was again a highly significant difference between the number of protected chickens in G1 and G2 controls. At this time, about 78% of the vaccinates were protected against vvIBDV challenge, while 85% of the unvaccinated controls were severely affected.

While the EP 0587 does not apply to conventional chicks, the challenges were designed on the model of this EP but delayed in order to cover the maximal age susceptibility of broilers to challenge (3 to 6 weeks of age) and taking into account the decay of maternally derived antibodies.
Vaxxitek HVT + IBD vaccine induced an equal or even better immunity than the classical vaccine in presence of maternally derived antibodies. This indicates the potency of Vaxxitek HVT + IBD as alternative to classical live vaccines. The field study was well described.

The potency and the duration of immunity of Vaxxitek HVT + IBD vaccine after *in ov*o vaccination of conventional chickens at 18 days of embryonation in field conditions has been demonstrated against a vvIBDV challenge. Immunity has been shown to persist at least as long as the economic life span of broilers.

Vaxxitek HVT + IBD vaccine – Recombinant live vaccine vHVT013-69 - Field trial – Potency against Marek's disease challenge after in ovo vaccination in broilers.

Objective: to study the potency of Vaxxitek HVT + IBD vaccine administered *in ovo* in field conditions.

Methodology: more than 60,000 eggs were included in the study, divided into two flocks which were successively bred in two conventional broiler Farms. The potency was assessed by experimental challenge with a virulent strain of Marek's disease (MD) virus carried out in Merial confined animal facilities.

For each flock, two groups were set up:

G1: Broilers vaccinated with 3 Vaxxitek HVT + IBD *in ovo.*
G2: Control broilers injected *in ovo* with sterile diluent.

- The *in ovo* injections were carried out at the hatchery in eggs of at least 18 days of embryonation, with an apparatus adapted to the *in ovo* vaccination of high numbers of eggs. The day of hatch was considered as Day 0 (D0) of the trial for each flock.
- For each flock, a MD challenge was carried out on D9 by intraperitoneal injection of virulent MD strain. There were 30 birds challenged in each G1 and G2.
- The birds were observed for at least 70 days post-, then euthanased and post-mortemed for signs of Marek's disease (hypertrophy and/or tumour formations on main organs and/or nerves). Any chicken dying prior to the end of the trial was necropsied to confirm infection with MD virus.
- A MD serological follow-up was also carried out in the remaining birds from G1 and G2 reared in the field by immunofluorescence (from hatching to a few days before slaughter).

Results:

- Zootechnical data showed no differences between G1 and G2 groups, for the two flocks.
- Serology results confirmed that Vaxxitek HVT + IBD was administered in the presence of high MD antibody titres at day-old. The number of positive sera for MD decreased afterwards in both groups, but other trials had shown late seroconversion to MD in the same model.
- Challenge: For each of the two challenges, very similar results were obtained in the two flocks, which allowed to assume the repeatability and the reliability of the data collected.

- The raw protection of the Vaxxitek HVT + IBD vaccinates (G1) was 87% and the relative protection score in G1 as compared to the conventional controls (G2) was 71%.

With regard to the diluent volume to be used for subcutaneous and *in ovo* routes, appropriate wording has been provided in the SPC.

The Applicant has demonstrated that the high level of maternal antibody levels seen were typical of high antibody levels seen in the field situation.

Conclusions on efficacy

Efficacy with respect to IBD:

The minimal protective dose against a challenge using a virulent IBDV has been demonstrated in a potency test performed in accordance with the EP. A low dose was sufficient to protect birds against mortality, clinical signs and severe lesions of the Bursa of Fabricius, 14 days after vaccination. In another experiment, the minimal dose (1000 PFU) was shown to protect birds against mortality, clinical signs and severe lesions of the Bursa of Fabricius (gross lesions were still observed in some animals) 14 days after a challenge using a highly virulent strain of IBDV. These studies show an onset of protection of 2 weeks in the absence of maternally derived antibodies. An onset of protection of 3 weeks has been demonstrated by challenge in conventional chickens in presence of maternally derived antibodies. Antibodies are present two weeks after vaccination.

The duration of protection was illustrated by 2 studies. The first trial showed persistance of anti-IBDV antibodies and of HVT virus for at least 8 weeks after vaccination. In the second one, no mortality, clinical signs and lesions were observed following a challenge using a highly virulent IBDV performed 8 weeks after vaccination. The duration of protection against challenge is of 8 weeks. Antibodies are observed up to 19 weeks after vaccination allowing to claim a duration of protection of 9 weeks.

The absence of influence of maternally derived antibodies on serological response to vaccination was demonstrated in a study involving conventional chickens. Specific anti-VP2 and antibodies were detected 7 weeks after vaccination. In another study, a challenge (using a very virulent IDBV strain) performed 3 and 6 weeks after vaccination in the presence of anti-IBDV MDA in chicks showed protection against mortality and severe lesions. The demonstration of efficacy in the presence of passive antibody is of considerable interest and might give this type of vaccine an important advantage over classical ones. This is one of the strongest advantage of the product.

The results on compatibility with Rispens Strain vaccine (live Marek's disease vaccine) showed that although the compatibility seemed complete on long-term study, some early but transient interference cannot be completely ruled out. Indeed, a delay in serological conversion in anti-IBDV antibody titres was observed. Additional data were provided demonstrating that the Rispens Strain vaccine had no adverse effect on the efficacy of Vaxxitek HVT + IBD with regard to seroconversion against IBDV. A new trial showed prevention of mortality and reduction of lesions against a challenge performed 15 days after vaccination with a Marek's disease serotype 1 Rispens vaccine and Vaxxitek HVT + IBD, and prevention of mortality and lesions against a challenge performed 43 days after vaccination with the same vaccines. It can be concluded that there is no interference between the Marek's disease serotype 1 Rispens vaccine and Vaxxitek HVT + IBD vaccine regarding the efficacy against IBDV.

The absence of interference regarding the efficacy against Gumboro disease has been demonstrated when a Newcastle disease vaccine or a vaccine against infectious bronchitis is administered.

In a first field trial, the serological anti-IBD response to the product was compared to that obtained with a reference live IBD vaccine. In a second field trial, the efficacy of the vaccine was tested by serological testing and a challenge performed 20 and 37 days post vaccination using a very virulent

IBDV strain. In both studies, the serological response was lower than that recorded in laboratory trials. The challenge resulted in a reduction of lesions and of clinical signs in vaccinated animals.

In conclusion, the claim "to prevent mortality, to reduce clinical signs and lesions of Infectious Bursal disease" was justified.

Efficacy with respect to MD:

The minimal protective dose against Marek's disease was determined in a potency test according to the EP. The minimal recommended dose was shown to be 97% protective against mortality and lesions following a challenge performed 4 days after vaccination. The product is able to reduce mortality and lesions but does not totally prevent them.

The absence of interference with maternally derived antibodies has been demonstrated by challenge 9 days after vaccination. Due to poor susceptibility of older conventional chickens (128 days) to challenge, no duration of protection is proposed but it is accepted that a single vaccination is sufficient to provide protection during the risk period.

A compatibility study with a Marek's disease serotype 1 Rispens vaccine was performed. This trial showed reduction of mortality and reduction of lesions against a challenge performed 9 days after vaccination with a Marek's disease serotype 1 Rispens vaccine together with Vaxxitek HVT + IBD (at the minimum recommended vaccinal dose). From these study it can be concluded that there is no interference between the Marek's disease serotype 1 Rispens vaccine and Vaxxitek HVT + IBD regarding the efficacy against a very virulent serotype 3 Marek's disease virus.

In conclusion, the efficacy against Marek's disease as claimed by the applicant "to reduce mortality, clinical signs and lesions of Marek's disease" was supported.

RISK-BENEFIT ASSESSMENT

Vaxxitek HVT + IBD is a live vaccine based on the use of a recombinant turkey Herpesvirus (HVT) expressing the VP2 gene of the Infectious Bursal Disease (Gumboro disease) Virus (IBDV). The pharmaceutical form is a frozen suspension with solvent for injection. The indication is for the active immunisation of day-old chickens as follows:

- To prevent mortality, to reduce clinical signs and lesions of Infectious Bursal disease.
  The onset of protection is from 2 weeks and the protection extends to 9 weeks.

- To reduce mortality, clinical signs and lesions of Marek's disease.
  The onset of protection is from 4 days. A single vaccination is sufficient to provide protection during the risk period.

The Applicant has performed laboratory and field trials to support the claims of the product. All studies have been performed using the minimal guaranteed dose of the product (3.0 log10 PFU) administered in the target species.

The product contains an organism within the meaning of Article 2 (1) of Council Directive 90/220/EEC. Written consent from the competent authorities from France to the deliberate release into the environment of the GMO for the purpose of research and development was provided. The data supplied by the Applicant for a risk analysis and complemented by available scientific data are based on the fact that a) the recombinant strain is derived from a widely used vaccine strain and b) no modification of the biological properties of the strain could be evidenced or can be foreseen. It can be considered that Vaxxitek HVT + IBD does not show any documented clinical or epidemiological safety risk. Moreover, based on current scientific knowledge and virological concepts, no foreseeable risk associated to its use under the conditions described was identified.

In contrast to live IBD vaccines, highly susceptible to MDA, Vaxxitek HVT + IBD does not show any interference with MDA. It can be thus administered to day-old chickens, without presenting any safety problem or inducing an immunosuppressive effect. In addition, all biological and immunological properties of the parental strain were retained. As a live vaccine, the product is excreted from vaccinated birds and may spread to turkeys. Safety and reversion to virulence trials have shown that the strain is safe for turkeys. However, precautionary measures have to be followed in order to avoid direct or indirect contact between vaccinated chickens and turkeys.

The use in breeding birds and birds in lay is contraindicated. No information is available on the safety and efficacy from the concurrent use with any other vaccine, except Merial live vaccines against Marek's disease, Newcastle disease and Infectious bronchitis. It is therefore recommended that no other vaccine than these should be administered within 14 days after vaccination with the product.

The Applicant has presented an approved packaging proposal for the particular storage conditions for this product and has provided sufficient assurance that differentiation of the vaccine from others contained in the same tank is possible.

Vaxxitek HVT + IBD is a frozen vaccine to be diluted with an aqueous diluent. In order to recommend a new route of injection (*in ovo* route), the Marketing Authoirsation Holder has proposed to increase the minimum titre from 3.0 log10 PFU/dose to 3.6 log10PFU/dose, based on the efficacy results. The maximum titre per dose remains unchanged. The stability of the vaccine in liquid nitrogen remains also unchanged. An overage was added as a security. The minimum release titre per dose has thus been set.

Apart from this modification, there is no other quality change compared to the Vaxxitek HVT + IBD vaccine already authorised in the EU.

The safety of the product has already been approved for the subcutaneous route of administration. The Applicant asked previously for scientific advice in order to provide appropriate data regarding the demonstration of safety by *in ovo* route. The safety of the vaccine has been evaluated in SPF chickens administered a maximal dose of the vaccine *in ovo*. The study provided supports the safety of the vaccine when administered *in ovo*. The choice of statistical approach (including the number of chickens used) was justified.

A recommendation in the SPC was included that "in the absence of specific studies, no other vaccines should be administered concurrently with the product by the *in ovo* route"

The efficacy of *in ovo* vaccination against infectious bursal disease (Gumboro disease) has been demonstrated in laboratory conditions in SPF animals against a challenge performed with a very virulent strain of IBDV. In this study the protective dose has been calculated and is lower than the minimum recommended dose of 3.6 log10 PFU. The onset of protection is 2 weeks after birth. The results allow the indication of active immunisation of 18 days embryonated eggs to prevent mortality and reduce clinical signs and lesions of infectious bursal disease. Based on serological data and correlation between serology and protection against challenge, the duration of protection is of 9 weeks.

A field study was performed where eggs were vaccinated in the presence of high maternal derived antibodies. The Applicant provided evidence that the maternal antibody levels in the field study were typical of high antibody levels seen in the field situation. Protection against challenge was demonstrated at 3 and 6 weeks of age.

For Marek's disease, a study performed using SPF eggs according to monograph 0589 determined a minimum protective dose which is lower than the minimum recommended dose of 3.6 log10 PFU. The onset of protection is of 7 days after *in ovo* vaccination that corresponds to 4 days after birth. The results allow the indication of active immunisation of 18 days embryonated eggs to reduce mortality, clinical signs and lesions of Marek's disease.

A field trial was performed. The efficacy of the vaccine against Marek's disease administered *in ovo* in the presence of high maternally derived antibodies has been demonstrated by challenge performed in 9 day-old chicks.

Immunity has been shown to persist at least as long as the economic life span of broilers.

As a conclusion, the results of laboratory and field studies regarding protection are fully compatible with those obtained with the subcutaneous administration of the vaccine to day-old chicks with regard to Gumboro disease and Marek's disease.

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