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SAFETY OF BOVINE VACCINES
Guideline for studies with inactivated vaccines

Guía n° 4 - G.B

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1. INTRODUCTIÓN

Inactivated bovine vaccines are basically composed of an antigenic element: either whole viruses or bacteria, or fractions of either, or toxoids, presented as one way or multivalent way and an adjuvant component (diverse principles in water solution or emulsion). Unlike pharmaceutical medicinal products, the formula for vaccines is not entirely definable, due to the complex composition of viruses and bacteria. Also, normalized potency trials for various bacterial vaccines (Leptospirosis, Clostridial diseases, Pasteurellosis, etc.) are challenged in laboratory animals; therefore, it is probable that those vaccines have not been tried in bovines during their development. For all the above mentioned, e well documented safety test in bovines is an ineludible step for every new vaccine applying for a license.

Continuous progress in the harmonization of international standards allows diminishing repetition of safety studies at the target species, avoiding the conduct of the same study in various countries. Thus, not only research costs but also the number of animals needed is reduced, taking into consideration the principles of animal welfare.

This guideline has been elaborated based on norms currently in force both in the American countries and the European Union, with the purpose of providing unified concepts that allow the acceptance of safety data by the regulatory authorities.

2. OBJETIVE

The aim of this guide es to provide recommendations for the conduct of studies that evaluate the safety of final formulations of inactivated vaccines for bovines. The document applies to batches at laboratory scale (experimental vaccines), at pilot scale and vaccines for registry, grouped under the acronym LPR.

3. SCOPE

The scope of application of this guideline is limited to evaluations of the health condition and welfare of bovines, who are subjected to a protocol of vaccination in accordance with the conservation and administration indications specified by the manufacturer. Recommendations at this guideline are addressed at the laboratories that manufacture inactivated vaccines for bovines, at the steps of development and pilot batches prior to the product license application and also at the evaluations of the finished product that may be requested. The guidance is not intended to cover safety studies conducted as part of post-approval batch release requirements, since these are conducted in laboratory animals (9. Cf., 113.33 and 113.38) or through documentary demonstration of manufacturing consistency parallel to studies in bovines (VICH. GL 50).

4. TERMS Y DEFINITIONS

- **Adverse Effect:** Any unfavorable observation suspected to be related to the LPR under investigation.

- **Adverse Event:** Any unfavorable and unintended observation which occurs after the use of a LPR, whether considered or not to be product related.

- **Class**: Subset of bovines that present certain characteristics in common such as age, reproductive status and/or use (calf, heifer, dairy cow, bulls, etc.).

- **Dosage:** Volume o (ml), frequency and interval between doses.

- **Field Safety Study**: Clinical study conducted using the vaccine under actual marketing conditions and following manufacturer´s indications to assess safety in the target animal.

- **Good Clinical Practices (GCP). -** Application of standardized procedures for the design, conduct, data recording, analysis and reporting of clinical studies. Adherence to the standardized procedures provides assurance that the data and reported results are complete, correct and accurate.

- **Good Laboratory Practices (GLP). -** Application of standardized procedures for the design, conduct, data recording, analysis and reporting of non-clinical studies. Adherence to the standardized procedures provides assurance that the data and reported results are complete, correct and accurate.

- **Laboratory Safety Study:** Clinical study conducted with the LPR in controlled conditions and following manufacturer´s indications, to assess safety in bovines. They are conducted before Field Safety Studies.

- **Masking/ Blinding:** A procedure to reduce potential study bias in which designated study personnel are kept uninformed of the treatment assignment.

- **Negative Control:** Healthy animals that are untreated or which receive placebo.

- **Pilot Batch**: A batch of a vaccine manufactured by a procedure fully representative of the one to be applied at commercial scale. Manufacturing methods should be identical, except for the scale of production.

- **Positive Control:** Healthy animals that are given a similar vaccine, which is normally registered in the country where the study is conducted. The product is chosen by the manufacturer and it is indicated for the same disease and target species claimed for the LPR under evaluation.

- **Production Batch:** A batch of a vaccine manufactured in the production facility where the vaccine will actually be manufactured by the method described in the application for registration.

- **Protocol:** A document that fully describes the objective(s), design, methodology, statistical considerations and organization of a study. The document is signed and dated by all the responsible persons taking part in the assay. The protocol may also include the background and rationale for the study, but that information may also appear in other study protocol-referenced documents. The term includes all protocol amendments.

- **Safety**.- In this guidance, the term safety (in Spanish *seguridad*) is used as a synonym of **innocuity,** term defined by the CAMEVET glossary as: "*Property of a veterinary product that indicates that its correct administration at the target species will not determine the presence of adverse effects in a statistically significant proportion*". In some documents from Spanish-speaking countries, the term ***tolerancia*** is also used with the same

meaning (MAPA Brazil, 2008 and SENASA Argentina, 2006).

5. GENERAL RECOMMENDATIONS

In order to plan a safety study using vaccines in bovines, the available information on several aspects, such as the type of vaccine, the nature of adjuvants, excipients, dose and proposed usage regimen, claims, previous usage history of similar products, target species class, and breed, need to be borne in mind; as well as all other data on safety that has been reported during the steps of development of the product. The mentioned data are important to support the design of the safety studies, defining the critical parameters that need to be examined.

The data from safety studies on combined vaccines may be used to demonstrate safety of vaccine(s) from the same manufacturer(s) containing less amount and/or a lower concentration of antigenic fractions, provided the remaining components are identical in each case and it is only the number of antigens what decreases in future formulations. Adverse events must be described and included in the final report, and determination of causality for the adverse event attempted.

6. GUIDELINE FOR THE CONDUCT OF STUDIES

The following recommended procedures refer to Laboratory Safety Studies, Field Safety Studies and Reproductive Safety Studies.

6.a) STANDARD PROCEDURES

Safety studies in bovines should be performed and managed in accordancde with Good Laboratory Practices (GLP), Good Clinical Practices (GCP) and internationally accepted animal welfare regulations.

6.b) LABORATORY SAFETY STUDIES

Laboratory safety studies are the first step in the evaluation of safety and provide basic information for the second stage, field studies.
The animals should be in the age, sex and class proposed by the vaccine label. Treated and control animals should be managed similarly and their environmental conditions should be the same. Every animal should be unequivocally and individually identified.
Facilities should be adequate for the purpose of the study and conform to local animal welfare regulations. Animals should enter study facilities one week before the initiation of the study, to acclimatize. Any sanitary treatment should be completed and reported before initiation of the study. Reduction or elimination of suffering during the study is essential. Euthanasia and necropsy of moribund animals is recommended.
Essential parameters to be evaluated for safety are: clinical observation of the animals, local and systemic reactions related to the vaccine and its resolution, as well as the effects of the vaccines on reproduction, when applicable. Study

protocol should be detailed and include the appropriate sheets for data collection.
Complementary tests, such as hematology, blood chemistry, necropsy or histological examination may be required. Where these tests are conducted in a subset of animals, these animals should be randomly selected with an adequate sampling rate before study initiation to avoid bias, unless otherwise justified. Samples should be properly selected, so that, in case of unexpected reactions or results, the cause of the problem observed may be identified.
The personnel participating in the studies should be masked (blinded) to treatment identification to minimize bias.
Working protocol may vary if applicable.

- One Dose and Repeat Dose Test

  One Dose or Repeat Dose Studies should be conducted with the LPR containing the maximum declared antigen concentration, or in case this is not specified, with a multiple of the minimum antigen concentration.
  For vaccines that require primary vaccination series followed by booster vaccination (2^{nd} dose), the interval applied should be the shortest of the vaccination regime recommended by the manufacturer. For practical reasons, the interval between administrations may be reduced up to 14 days.
  In general, 8 animals per group should be used, unless properly justified. The most sensitive class, age and sex proposed on the label should be used.
  If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If one route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study.

- Overdose Test

  When considered appropriate, an overdose of vaccine, for each of the indicated routes and for the subset of animals indicated by the manufacturer should be administered. For inactivated vaccines, the overdose study is established as an administration of the double of the indicated dose, in only one application. (E.P. 7.0, 2013)

- Data Collection

  General clinical observations should be made every day for 14 days after each administration, trying to maintain the same observation time. In addition, other relevant criteria such as rectal temperature or performance measurement (weight gain, dairy production, etc.) are to be recorded within this observation period with appropriate frequency. Injection sites should be examined daily or at other justified intervals by inspection and palpation and measurement for a minimum of 14 days after each administration of the LPR being tested. When injection site adverse reactions are present at the end of the 14 days observation, the observation period should be extended until clinically acceptable resolution of the

lesion has occurred or, if appropriate, until the animal is euthanized and histopathological examination is performed.
When previous information on similar vaccines is available, it is convenient to set acceptance criteria (hyperthermia up to certain time, acceptable size of local reactions, etc.). When designing recording sheets, it is useful to anticipate the annotation of the length of eventual systemic and local reactions, as well as their way of resolution

- Statistical Analysis

In laboratory studies the safety implications are best addressed by applying descriptive statistical methods to the data. Tables and descriptive text are common methods of data summarization; however, it may also be valuable to make use of graphical presentations in which patterns of adverse events are displayed both within treatments and within individual animals. In field studies, if applicable, selection of the general form for a statistical model and the factors to be included in the model will depend on the nature of the response variable being analyzed and the study design. Regardless of the methods chosen, the process and steps used to conduct any statistical evaluations should be described. The outcomes of the data analysis should be clearly presented to facilitate evaluation of potential safety concerns. The terminology and methods of presentation should be chosen to clarify the results and expedite interpretation.
Although there may be interest in the null hypothesis of no difference between treatments, study design constraints limit the statistical power and discriminatory ability of these studies. Under these conditions, statistical analysis alone may not detect potential adverse effects and provide assurance of safety. A statistically significant test does not necessarily indicate the presence of a safety concern. Similarly, a non-significant test does not necessarily indicate the absence of a safety concern.
Therefore, results should be evaluated based on statistical principles but interpretation should be subject to veterinary medical considerations.

6.c) REPRODUCTIVE SAFETY STUDIES

Examinations of reproductive performance of animals in breeding animals must be considered when data suggest that the starting material from which the product is derived may be a risk factor. Laboratory Studies together with Field Safety Studies are required to support use in breeding animals. If Reproductive Safety Studies are not performed, an exclusion statement must be included on the label, unless a scientific justification for absence of risk for use of the LPR in breeding animals is provided.

For examination of reproductive safety, animals appropriate for the purpose of the study will be vaccinated with at least the recommended dose according to the vaccination scheme indicated. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If one route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study. Generally, 8 animals per group should be used unless

otherwise justified. The animals should be observed for a period appropriate to determine reproductive safety, including daily safety observations. Exceptions should be justified. A control group should be included.
Vaccines recommended for use in pregnant animals must be tested as described above in each of the specific periods of gestation recommended for use by the manufacturer. An exclusion statement will be required for those gestation periods not tested. The observation period must be extended to parturition, to examine any effects during gestation, parturition or on progeny.
Exceptions should be justified.
When scientifically warranted, additional studies may be required to determine the effect(s) of LPR on semen. The observation period should be appropriate for the purpose of the study.

6.d) FIELD SAFETY STUDIES

Where epidemiology of the disease to be prevented and husbandry practices are similar between regions or countries, international data from field studies may be used, as long as it is accepted by the regulatory authorities. The manufacturer is responsible for ensuring that field studies are conducted under animal husbandry conditions representative of those regions and countries in which authorization is sought. Appropriate health authorizations must be obtained and consultation with regulatory authorities regarding study design prior to conduct of the studies is recommended.
If a label indicates use in breeding animals, appropriate field safety studies need to be performed to show the safety of the LPR in field conditions.

- Animals and study sites

  Bovines should be in the age, range and class intended for treatment as indicated in the proposed labeling. Previous serological status may be considered, although it is not a restrictive condition. A control group should be included.
  Two or more different geographical sites are recommended to conduct safety studies. The recommended dosage(s) and route(s) for vaccination should be used. The studies should be conducted using representative production batch(es) of the vaccine.

7. FINAL REPORT

Procedures must be recorded in working protocols, following Good Clinical Practices (GCP). Observations should be made over a period of time appropriate for the LPR and its adverse events should be documented and included in the final report. Reasonable attempts should be made to determine causality for the adverse event(s).

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