The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NOTE FOR GUIDANCE:

HARMONISATION OF REQUIREMENTS FOR EQUINE INFLUENZA VACCINES

SPECIFIC REQUIREMENTS FOR SUBSTITUTION OR ADDITION OF A STRAIN OR STRAINS

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INTRODUCTION

Equine influenza has remained among the main acute contagious respiratory diseases of horses worldwide. Equine influenza is represented by two subtypes: Influenza A/ equine 2 virus (H_3N_8) which is the most important cause of respiratory diseases in the horse, and Influenza A/ equine 1 virus (H_7N_7) which is still circulating subclinically but is almost considered as extinct.

However, a divergence in the evolution of A/equine 2 (H_3N_8) viruses had occurred since 1987 and 2 families of viruses are now circulating. These were designated European-like and American-like, although representatives of both families had been isolated in both continents (1). There is increasing evidence from field studies that antigenic drift in the gene coding for the Haemagglutinin (HA), which is the major surface protein of these influenza A strains, eventually renders vaccine strains obsolete and is likely to compromise vaccine efficacy (1,2,3).

A formal reporting mechanism on antigenic/genetic drift or shift of equine influenza viruses and a vaccine strain selection system has been set up, so that vaccine manufacturers and regulatory authorities are informed of the potential need to update vaccine virus strains.

An Expert Surveillance Panel, including representatives from 3 WHO Reference Laboratories and from 3 OIE Reference Laboratories reviews every year the epidemiological and virological information and makes recommendations about suitable vaccine strains. These recommendations are published annually by the OIE in its *Bulletin* (4). As antigenic drift in equine influenza occurs at a slower rate than in human influenza, it is considered that a regular update of the strains could be necessary every three to five years.

The development of effective vaccines can now be facilitated by the availability of reliable in vitro assays such as:

- Single Radial Diffusion (SRD) to measure vaccine bulk antigen content in terms of hemagglutinin (HA) content
- Single Radial Haemolysis (SRH) to measure serological responses.

For in-process controls, SRD provides a reliable method of measuring haemagglutinin content of equine influenza bulk antigens, although it cannot be used on final adjuvanted products. Use of SRD tests is therefore limited to the in-process control of adjuvanted vaccines. SRD tests can provide a great improvement on the Chick Cell Agglutination (CCA) test as it is not susceptible to wide test variation and measures immunologically active HA (5).

SRH is a sensitive and reproducible method for measuring antibody to haemagglutinin.

These guidelines are to provide a basis for rapid response to changing epidemiological situations within current subtypes (antigenic drift). There are a number of possibilities that are foreseen. These include the following:

1. Substitution of one strain
2. Substitution of two strains of one of the subtypes present in the original formulation
3. Substitution of two strains one to each of the different subtypes present in the original vaccine formulation

4. Addition of one strain of one of the subtypes present in the original vaccine formulation
5. Addition of strains one to each of the different subtypes present in the original vaccine formulation

In preparing these guidelines, the following assumptions have been made:

A) It is not expected that manufacturers will modify their vaccine to exclude A/ equine 1 virus strains, as there is insufficient evidence to justify such a change.

B) In the case of adding strains, it is not expected that there will be
   i) a decrease in the antigen content of the original strains,
   ii) any change to the method of production of the original strains (other than increasing the degree of concentration applied by the currently approved method), or
   iii) a change in the quantity of adjuvants or ratio of the volume of antigen to adjuvants.

C) It is not expected that manufacturers will add two new strains of the same subtype to their vaccines at the same time.

If conditions (B) and (C) do not apply to the changes being made, then additional data to that described below will be required.

If major antigenic shift occurs, these guidelines would not be applicable. Emergency measures would be required.

SPECIFIC REQUIREMENTS

A new outbreak associated with a possible breakdown of existing vaccines may require a change in the formulation of such vaccines. It is expected that manufacturers will wish to make such changes in response to evidence of an antigenic drift and on the need for such a change from the report and recommendation from the Expert Surveillance Panel.

Equine influenza vaccines are well known, and it is unlikely that the replacement of one strain by another would lead to such substantial changes so as to justify a new full set of safety and efficacy tests to be carried out. In addition, there is a need to consider reduction of the number of animals used in the testing of medicinal products whenever possible.

Therefore, provided there have been no or few adverse reactions with the previous formulation, a two-fold approach is proposed for the testing of the new formulation:

(a) data and documentation from the original dossier would be accepted for those parts which remain unchanged.

(b) where necessary, the analytical, safety and efficacy sections of the original dossier would need to be amended and new additional data generated as described below. The three expert reports will also require updating from those submitted for the previous formulation.

ANALYTICAL TESTS

II.A Qualitative and Quantitative particulars of the constituents

Data on the new strain shall be provided.

II.B Description of the method of preparation of the finished product

The method of manufacture of the new strain shall be described and the production stages from the monovalent bulks to the final product updated.

Inactivation kinetics data will have to be submitted for the new strain in accordance with the principles laid down in the European Pharmacopoeia monograph on Inactivated Equine Influenza Vaccines.

When adding a strain there must be a clear description of any consequential changes to the preparation of the existing bulk antigens (e.g. concentration steps) and of the method of blending to include the additional strain(s).

II.C Production and control of starting material

Information on the rationale for substituting the new strain for the old one shall be included. The source of the recommended strain will be published by the OIE, but manufacturers will have flexibility to select local strains if justified. In such a case, manufacturers should document the relationship between the recommended strain and the recommended-like strain they propose to use.

The recommended strain will have been characterised by genetic and antigenic analysis by laboratories of the Expert Surveillance Panel.

If manufacturers wish to use a recommended-like strain, they should prove that their Master Seed Virus is antigenically similar to the antigenically characterised recommended virus held at the Reference Laboratory by contracting Reference Laboratories to undertake comparative tests or by providing all the reagents for this purpose. For recommended-like strains, reagents used in the testing procedure shall be provided by the manufacturer on request to enable the competent authorities to arrange for check tests to be carried out.

Details of the preparation of the new master seed and working seeds, the tests carried out on it (e.g. identity, extraneous agents, sterility, mycoplasma) and the results of these tests shall be provided as required by Council Directive 81/852/EEC.

In particular, the Master Seed of the new strain shall be shown to contain only the recommended strain or the recommended-like strain chosen by the manufacturer. A suitable method shall be provided to identify the new strain and to distinguish it from related ones.

Furthermore, if applicants wish to produce vaccine in mammalian cells which may result in host cell selection phenomena, they shall demonstrate that their working seed viruses are antigenically similar to the master seed and relevant to the recommended strain(s).

IID and IE Control tests during production and tests on the finished product

- General characteristics of the finished product

Results of general tests carried out on one or more batch of the finished product shall be included in this section as required by Council Directive 81/852/EEC.

- Identification and assay of the active ingredients

The following should be undertaken to measure and standardise the antigen content and the biological activity of the finished product:

1. SRD tests or other suitably sensitive validated immunochemical test shall be used for the in-process measuring of the antigenic content of each bulk and the limits set as required to prepare a satisfactory batch of vaccine.

2. SRH tests or other suitably sensitive validated immune response tests should be carried out to determine the antibody response in guinea pigs immunised with the finished product and the limits set in accordance with those required for a satisfactory batch of vaccine and taking account of available validation data for the test.

Safety tests on each batch of the finished product will have to be carried out in accordance with the principles laid down in the European Pharmacopoeia monograph on Inactivated Equine Influenza Vaccines.

IIF Stability tests

It is expected that the shelf life can remain unchanged from that previously agreed for the vaccine providing on-going real-time stability studies are undertaken with the new formulation and results reported at regular intervals, but no later than the deadline agreed upon, to the competent authorities (Post-authorisation commitment).

These on-going studies on the new formulation are required on at least the first 3 consecutive batches stored and tested until 3 months beyond the required shelf-life.

In the meantime, while data is gathered to justify the shelf-life, batches released should be tested at intervals to provide assurance that the potency is still satisfactory (e.g. for a 2 year shelf-life, the batch would be tested after 12 and 18 months). Any batch failing the potency tests before the end of its shelf-life would be withdrawn and the shelf life reassessed.

SAFETY TESTS AND EFFICACY TESTS

Pre-marketing requirements

III- Safety

To provide data on the safety of the product, the following should be undertaken with studies carried out to address all the requirements of the EU guidelines and directives (e.g. use of a batch or batches of maximum potency; conduct appropriate post-vaccination monitoring).

1. Substitution of one or two strains:

The horses vaccinated for the efficacy study on the new formulation should be monitored for systemic and local reactions to the vaccination and the results presented.

2a. Substitution of one or two strains:

Careful note must be taken of the reactions observed in the horses used in the pre-authorisation batch safety test. The rectal temperature of the horses should be monitored before and after vaccination. Local reaction should be carefully monitored and recorded. The detailed results should be presented together with information on comparison with historical data from tests carried out on the existing formulation.

2b. Addition of one or two strains:

The pre-authorisation batch safety test has to be modified as follows:
The test should be carried out in young animals of the minimum age recommended for vaccination, unless a justification is given to use older animals. These animals should be seronegative or have no more than low levels of maternal antibodies to Equine Influenza viruses. In addition to the double (in one site) and single dose vaccination schedule, required by the EP monograph, a third vaccination should be given two weeks later to test the safety of a repeat dose. Careful note must be taken of the reactions observed in the horses. The rectal temperature of the horses should be monitored before and after vaccination. Local reaction should be carefully monitored and recorded. The detailed results should be presented together with information on comparison with historical data from tests carried out on the existing formulation.

IV- Efficacy

1. Tests in guinea pigs

a. Substitution of one or two strains:

To provide information on the immunogenicity of the new strain and its interaction with the existing remaining strain(s) and/or other antigens (e.g. tetanus), SRH tests or other suitable validated tests shall be carried out on the one hand with sera of guinea pigs immunised with the new combination of the strains and on the other with each single strain.

b. Addition of one or two strains:

The investigation with the new combinations should include the following comparison with the original vaccine formulation.

i) The antibody response in guinea pigs immunised with the new combination of the strains should not be less than that obtained from the original vaccine formulation.
ii) The antibody response in guinea pigs immunised with the new combination of the strains should be shown to contain strain specific antibodies stimulated by the new strain. This should be demonstrated by testing sera in an SRH test or another suitable validated test after absorbing out specific and cross-reacting antibodies against the original strains of the same subtype.
iii) The antibody response in guinea pigs immunised with the new combination of the strains should be shown to continue to contain strain specific antibodies stimulated by the original strain. This should be demonstrated by testing sera in an SRH test or another suitable validated test after absorbing out specific and cross-reacting antibodies against the new strains of the same subtype.

2. Tests in horses:

a. Addition of one or two strains:

In addition to testing the new formulation in horses, each new antigen should be shown to be immunogenic. For each new strain a monovalent vaccine containing the antigen content of the new strain or strains to be used in the finished product should be inoculated into at least four seronegative horses as per the Immunogenicity section of the European Pharmacopoeia monograph on Inactivated Equine Influenza Vaccines.

b. Substitution and addition of one or two strains:

The new formulation should be tested in at least six seronegative horses, vaccinated as per the Immunogenicity section of the European Pharmacopoeia monograph on Inactivated Equine Influenza Vaccines and shown to be in compliance with the requirements. The antibody levels stimulated shall be not less than those achieved with the vaccine in the original license application. The serological assay shall be standardised using the European Pharmacopoeia/OIE reference sera. The results of this testing shall then be discussed and compared to historical data from the previous formulation.

In the light of results from such studies, it can be stated that the vaccine contains the new strain and that it induces a serological response to it.

Post-authorisation commitments

Manufacturers are required to be involved in on-going trials and field monitoring of equine influenza vaccines and to present the results to the competent authorities.

The purpose of such trials is to verify:

- the tolerance or incidence of adverse reactions (as part of normal pharmacovigilance studies);
- the immunogenicity of the vaccine, i.e. the level and duration of antibody responses (at least 10 horses).

Sera should be collected at intervals from horses vaccinated according to the recommended schedule. Antibody to the HA of viruses included in the vaccine should be measured by SRH or a suitably validated test. If the horses are not kept under controlled conditions with unvaccinated in-contact control animals, freedom from infection during the period must be confirmed by screening for infection specific antibodies (6).

The field trials are carried out by the manufacturers, who will have to forward the results, as soon as they have been obtained, but no later than the deadline agreed upon, to the competent authorities.

1. Daly, J.M., Lai, A.C.K., Binns, M.M., Chambers, T.M., Barrandeguy, M. and Mumford, J.A. (1995) Recent worldwide antigenic and genetic evolution of equine H3N8 influenza A viruses. Journal of General Virology, 77, 661-671.

2. Mumford, J.A., Wood, J. (1993) Conference report on WHO/OIE meeting: Consultation on newly emerging strains of equine influenza. Vaccine, 11, 1172-1175.

3. Mumford, J.A., Chambers, T., and Wood, J. (1996) Consultation of OIE and WHO Experts on Progress in Surveillance of Equine Influenza and Application to Vaccine Strain Selection.

4. OIE 64th General Session. Standards Commission Report, February 1996.

5. Wood, J.M., Schild, G.C., Folkers, C., Mumford, J. and Newman, R.W. (1983) The standardisation of inactivated equine influenza vaccines by single-radial immunodiffusion. Journal of Biological Standardisation, 11, 133-136.

6. Birch-Machin, I., Rowan, A., Pick, J., Mumford, J.A. and Binns, M.M. (1996) Expression of the nonstructural protein NS1 of equine influenza A virus: Detection of anti-NS1 antibody in post infection equine sera. (Submitted)