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Committee for Medicinal Products for Veterinary Use (CVMP)

Guideline on the procedure to be followed when a batch of a vaccine finished product is suspected to be contaminated with bovine viral diarrhoea virus

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The guideline, originally adopted by CVMP in March 2008, was revised to remove the provision for an in vivo test, with the view to ensuring the current best practice with regard to implementation of 3Rs (replacement, reduction and refinement) principles.

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1. Introduction (background)

Starting materials of animal origin, including bovine serum are still essential ingredients of the cell culture media used in the production of many immunological veterinary medicinal products. Different risks are associated with the use of such starting materials. The presence of extraneous agents in bovine serum certainly represents a major risk to the quality of the finished product. One of the specific risks associated with the use of bovine serum is the contamination of the finished vaccine with Bovine Viral Diarrhoea Virus (BVDV).

The challenge when suspecting contamination of a vaccine batch with a BVDV is the confirmation of this contamination. It is therefore important to agree on an approach to this confirmation of contamination, which can be followed and mutual recognised by all the Competent Authorities.

2. Scope

The aim of this guideline is to outline the procedure to be followed by the Competent Authorities when a batch of a vaccine is suspected to be contaminated with BVDV. Considering the risk of BVDV in bovine serum, the highest risk will be with live and inactivated vaccines indicated for use in pestivirus susceptible species (bovine, porcine, ovine, caprine). Of these, the greatest risk is associated with the use of live vaccines in pregnant BVDV susceptible females.

3. Legal basis

This guideline has to be read in conjunction with the introduction and general principles and Title II of Annex I to Directive 2001/82/EC.

4. Definition of a suspicion

Contamination of a vaccine batch with BVDV (type 1 or 2) can be suspected when Bovine Viral Diarrhoea (BVD) or Classical Swine Fever (CSF) antibodies are detected in animals without vaccination or field infection history after the use of inactivated or live vaccines not containing BVDV vaccine antigens or in unexplained conditions (e.g. CSF diagnostic in pigs; not associated with a CSF epizootic or when a CSF outbreak is definitely excluded). Reproductive disorders (abortion, mummification, stillborn neonates etc.) may be associated with the use of a batch of vaccine contaminated with live BVDV in pregnant females susceptible to the virus.

The risk of non-specific amplification should be taken into account when selecting primers for detection of BVD genome by reverse transcription polymerase chain reaction (RT-PCR). Furthermore, the principles stated in the CVMP guideline on `Requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products' (EMEA/CVMP/743/00), which indicates clearly (4.3.1) that the applicant should be able to clarify whether or not any nucleic acid detected originates from infectious BVDV particles, should also apply.

The use of bovine serum containing inactivated BVDV particles should not (in some cases, albeit rare, serum is added as a stabiliser to the finished vaccine) induce any serological response in the animals inoculated with the finished product of the vaccine as the dilution of the bovine serum is too high during the manufacturing process. However, if the bovine serum contains live BVD viral particles, they will multiply in the cells used for the vaccine production and the viral burden will be high in the finished product.

- If a live vaccine is contaminated with live BVDV (type 1 or 2) and is administered to pregnant females (bovine, ovine, caprine, porcine), an iatrogenic disease may be induced in the foetuses which can be similar to BVDV, CSF or border disease. The disease could be of particular severity in the case of a vaccine contamination caused by a BVDV type 2 strain (haemorrhagic disease).
- If an inactivated vaccine is contaminated with BVDV (type 1 or 2) and is administered to bovines, a seroconversion may be induced, which depends on the concentration of the contaminant.

  In pigs, sheep or goats, a vaccine contaminated with BVDV may interfere with the diagnostic measures for CSF or border disease due to induction of BVD antibodies.

5. Detection of BVDV contamination

In vitro diagnostic methods are currently available which can usefully be used to detect BVDV contamination. The sensitivity and specificity of these tests need to be demonstrated. When PCR is used, the manufacturer should be able to clarify whether or not any nucleic acid detected originates from infectious particles.

In vitro tests can be used either as first choice or confirmatory tests to detect BVDV contamination of finished products. When BVDV contamination is suspected and PCR is used for the diagnosis, it is preferable to use a semi-quantitative RT-PCR with an internal control, which will allow quantification of the number of detected BVDV RNA copies in each sample. This should already allow discrimination between background signals and serious BVDV contamination. In order to determine if the detected sequences are the signals of infectious or non-infectious particles, a confirmatory *in vitro* test has to be carried out on the original sample.

As an example of a first choice or confirmatory (for PCR) diagnostic method to detect BVDV contamination, an *in vitro* test can be carried out as follows.

After neutralisation of the active ingredient(s) of the vaccine (in the case of live vaccines) with mono specific antiserum (free of BVD antibodies), the finished product has to be inoculated into cells sensitive to but free of BVDV.

It is advisable to incubate as many wells as possible (such as twenty wells on a 24 wells microtitre plate), and perform at least three passages of the cells inoculated. Monolayers are observed for the appearance of cellular changes caused by replicating cythopathogenic strains of BVDV. The presence of non-cytopathogenic strains of BVDV should be screened by using an immunoperoxydase monolayer/linked assay (IPMA/IPLA) or an immunofluorescent assay or PCR.

6. Measures to be taken

Measures have to be taken (alert, withdrawal of the contaminated batch) only when positive results are obtained with the *in vitro* test.