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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 (BVD) VIRUS

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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 (IVMPs). 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 (BVD) virus.

The challenge when suspecting contamination of a vaccine batch with a BVD virus 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 Bovine Viral Diarrhoea (BVD) Virus. 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 (4) and title II of the Annex I to Directive 2001/82 as amended.

4. DEFINITION OF A SUSPICION

Contamination of a vaccine batch with BVDV (type 1 or 2) can be suspected when 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 RT-PCR. Furthermore, the principles stated in the guideline on "Requirements and controls applied to bovine serum…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 bovine viral diarrhoea, classical swine fever 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 porcines, ovines or caprines, a vaccine contaminated with BVDV may interfere with the diagnostic measures for classical swine fever or border disease due to induction of BVD antibodies

  For example, after several injections of the vaccine, alleged CSF antibodies can be detected as the repeated administration of the contaminated vaccine induces the appearance of BVD antibodies which cross react with CSF antigens. This can interfere with CSF surveillance programmes based on serological sampling, particularly in breeders which are usually given numerous vaccines against several viral diseases e.g.: Aujeszky's disease, Parvovirus.

  In countries applying eradication or control programmes against BVD, interference with national surveillance programmes can also occur when cattle are repeatedly vaccinated with BVDV contaminated, inactivated vaccines such as IBR.

5. PROTOCOL TO BE APPLIED TO CONFIRM THE BVD CONTAMINATION

It is essential to be able to determine if the detected sequences are the signals of infectious or non-infectious particles. In this situation, a double test has to be carried out:

5.1 Diagnosis of BVDV contamination by PCR

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 molecules 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 second test has to be carried out on the original sample.

5.2 Diagnosis of BVDV contamination by other tests

In vitro and/or *in vivo* tests can be used to detect BVDV contamination of finished products. In principle, only *in vitro* tests should be used to detect BVDV contamination of live vaccines. If the results of the *in vitro* test is negative, either as first choice test or as confirmation of PCR, *in vivo* test should be avoided, unless justified. Repeated vaccine administration could be necessary to enable low-level BVDV contamination to be detected. Normally, if a vaccine, which contains a small number of BVD infectious particles, is administered to susceptible animals, BVDV antibodies should appear after the 1st or second injection. BVDV antibodies, especially with inactivated virus vaccines, can appear only after several injections of susceptible animals.

In vitro test: 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 sensitive cells, free of BVDV (such as BT cell line…).

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.

If it is possible to perform this *in vitro* test (complete neutralisation of the vaccinal strain, absence of the toxicity of the inoculum) then it is not necessary to carry out the following *in vivo* test.

In vivo test (Test to be performed only in exceptional circumstances): Inoculate 10 doses (in the case of live vaccine) or 2 doses (in the case of inactivated vaccines) to, at least, 5 BVD susceptible animals, free of specific antibody and antigen. Repeat vaccine administration at least 3 times (4 injections in total) every 2 weeks. Collect blood samples before the first injection and before each repeated injection to detect BVD antibodies and antigen.

It is important to remember that with inactivated vaccines contaminated with a low concentration of BVDV antigen, only in vivo tests with repeated injections may demonstrate possible interference with an eradication programme against BVD.

6. INTERPRETATION OF THE RESULTS OF THE APPLIED PROTOCOL

1) PCR - = no further tests. Product considered as non-contaminated.

2) PCR- or in vitro test - = no further test needed (unless justified: non optimal conditions to carry out the in vitro test, see above) at this stage. Product considered as non-contaminated.

3) in vitro + = no need for in vivo test. Product considered as contaminated even if the PCR is negative.

4) PCR+/in vitro test impossible to be carried out = the in vivo test is performed (in addition it is carried out in the case of risk analysis for interference with BVD eradication programme) to verify the possibility of a contamination.

5) PCR+/in vivo test + = Product considered as contaminated.

7. 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 (isolation of a BVD virus) and/or with the *in vivo* test (detection of BVD antibodies after the 1st and following injections and negative results before the 1st injection).

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