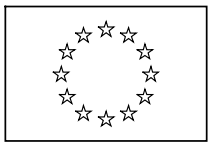
Sanco/C3/AH/R21/2000



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Health Opinions
Unit C3 - Management of scientific committees II; scientific co-operation and networks

Virus inactivation in bovine blood and blood products

Scientific Committee on Animal Health and Animal Welfare

Adopted 25 October 2000

Virus inactivation in bovine blood and blood products

Report of the

Scientific Committee on Animal Health and Animal Welfare

Contents

Prof. Dr J Van Oirschot, member of the Scientific Committee on Animal Health and Animal Welfare, acted as rapporteur for this report.

1. Request for opinion

The Scientific Committee on Animal Health and Animal Welfare was asked to respond to the following question:

Is the system currently contained in EC legislation (Chapter 7 of Annex I of Directive 92/118/EEC) of checking for neutralisation of five specific viruses the most appropriate to avoid the risk of spread of serious transmissible diseases or of diseases transmissible to man which could result from movement of this product, not only for bovine animals but also for other species which could carry the disease or become a focus of disease or a risk to public health?

The question relates only to bovine blood and blood products and does not include prion diseases[1].

2. Background

Bovine blood and blood products are often used for the production of biopharmaceuticals, e.g. vaccines. It is common knowledge that bovine blood products can be contaminated with viruses, and it is therefore important to ensure that these products do not contain such adventitious viruses. Various methods have been described for the inactivation of viruses in bovine blood, and in addition procedures to validate the inactivation process have been reported. However, a disadvantage of most inactivation approaches is that they affect the growth-promoting properties of bovine sera. In the EU directive 92/118/EC several treatments for virus inactivation are listed.

[1] See: Safety of ruminant blood with respect to TSE risks, and Opinion on specified risk materials of small ruminants, Scientific Steering Committee, 13-14 April 2000.

3. EU legislation

Directive 92/118/EEC deals with the importation of blood and blood products of ungulates into the European Union. Relevant parts of the directive state that:

Imports of blood products should be accompanied by a certificate, certifying that:

> the products originate in a third country in which no case of foot-and-mouth disease has been recorded within at least 24 months and no case of vesicular stomatitis, swine vesicular disease, rinderpest, peste des petits ruminants, Rift Valley fever, blue tongue, African horse sickness, classical swine fever, African swine fever, Newcastle disease or avian influenza has been recorded for 12 months in the susceptible species and in which vaccination has not been carried out against those diseases for at least 12 months. The health certificate may be made out according to the species of animal from which the blood products are derived,
>
> **or**
>
> in the case of the blood products derived from bovine animals, they originate in an area of a third country fulfilling the conditions set out in the first indent from which imports of bovine animals, their fresh meat or their sperm are authorized pursuant to Community legislation. The blood from which such products are manufactured must be from bovine animals from that area of the third country and must have been collected:
>
> - in slaughterhouses approved in accordance with EU legislation,
> - in slaughterhouses approved and supervised by the competent authorities of the third country. The Commission and Member States must be notified of the address and approval number of such slaughterhouses,
>
> **or**
>
> in the case of blood products derived from bovine animals, they have undergone full treatment guaranteeing the absence of pathogens of the bovine diseases referred to in the first indent.

4. Bovine blood and blood products and their risk as a vehicle for spread of virus diseases

Micro-organisms, including viruses, bacteria, and some parasites may spread thoughout the body's tissues by the blood stream. As a consequence, blood or blood products may contain micro-organisms. Bacteria, parasites and fungi can be removed from serum by filtration through 200 nm filters. Filtrates obtained after passage of broth cultures of mycoplasms through 220-nm membrane filters usually demonstrate a decline of 5 to 6 logs in CCU/ml over unfiltered broth cultures, wherease 100 nm filtrates are usually sterile (Tully, 1980). The contamination of blood and blood products with the above micro-organisms is not dealt with.

The table below lists some of the most important viruses that give rise to a viraemia in adult or foetal bovines:

DNA viruses	**Viraemia Adult(A)/foetal (F)**	**RNA viruses**	**Viraemia Adult (A)/foetal(F)**
Vaccinia	AF	Reo virus	A
Cowpox	A	Bluetongue virus	AF
Bovine papular stomatits virus	A	Epizootic haemorrhagic disease of deer virus	A
Lumpy skin disease virus	A	Bovine viral diarrhoea (BVD) virus	AF
Bovine herpesvurus 1 (Infectious bovine rhinotracheitis)	AF	Wesselsbron disease virus	AF
Malignant catarrhal fever	A	Parainfluenza-3	A
Bovine herpesvirus 4	AF	Rinderpest virus	A
Adenovirus	A	Vesicular stomatitis	A
Parvovirus	AF	Bovine ephemeral fever virus	A
		Bovine leukosis virus	A
		Akabane virus	AF
		Rift valley fever virus	AF
		Foot-and-mouth disease virus	A
		Bovine enterovirus	AF

Because foetal bovine serum and newborn calf serum are often used in the production of vaccines and may be used to produce other biopharmaceuticals, there is a risk, provided the blood is from countries where these viruses are present, of transmitting the above viruses to cattle and other animals and perhaps to man.

A complicating factor is that the products (e.g. vaccines), that harbour the blood or blood products in question are often injected. By this route the likelihood that a virus may cross the species barrier is greater than under natural conditions. This implies that requirements need to be even more stringent.

It has been stated that BVD virus is present in most if not all commercial-size batches of bovine sera (Anonymous, 1986). To guarantee absence of viruses the blood or blood product or serum must be treated in such a way that all relevant viruses are inactivated, irrespective of the method of inactivation. Inactivation of viruses is dependent on various factors, e.g. the properties of the virus (e.g. DNA or RNA, single stranded or double stranded genome, genome size, naked or enveloped), the virus titre, the medium in which viruses are suspended, the pH, and the salt concentration of the medium (Sullivan et al., 1971).

The virus titres per ml of blood or serum of individual bovines or bovine fetuses can vary between 10^1 and 10^8 infectious doses. In addition, some inactivation procedures are better suitable for one virus family than for another virus family (Thomas et al., 1981, House et al., 1990, Hanson and Foster, 1997).

5. Risk assessment of Bovine Viral Diarrhoea Virus in foetal calf serum

Bovine viral diarrhoea virus (BVDV) is the virus most often contaminating foetal bovine serum. The existing EC legislation can not prevent BVDV contaminated foetal bovine serum batches entering the market. In individual foetuses BVDV can be present in the blood up to titres of 10^6 $TCID_{50}$ per ml (Straver et al., 1983). There is a prevalence of 0.5 to 2% of persistently-infected calves (Houe, 1999), implying a somewhat higher percentage of persistently-infected foetuses. If a batch is composed of blood from 100 bovine foetuses and assuming that 5 out of these 100 are persistently-infected foetuses and assuming a titre for each of 10^6 $TCID_{50}$ per ml of foetal blood, then the BVDV titre in the batch will be $10^{4.7}$ $TCID_{50}$ per ml.

It has been described that a dose of 25 kilogray (=2.5 Mrad) gamma-irradiation inactivated 6 logs of $TCID_{50}$ of BVDV when spiked serum in 500 ml bottles was irradiated (Hanson and Foster, 1997, Daley et al., 1998). Based on the above, it may be stated that a commonly used virus inactivation procedure may profoundly minimise the risk for infectious BVDV contamination of foetal bovine serum. On the other hand, BVDV has still been found as contaminant of recent commercial vaccines, which indicates that absence of infectious BVDV can not yet be fully guaranteed. The probable presence of antibodies against BVDV in most foetal bovine serum batches may influence the detection of infectious BVDV before or after the application of viral inactivation procedures.

The above illustrates that more safeguards are needed to prevent the risk of BVDV contaminated bovine blood acting as a source of BVDV spread in the animal population.

6. Conclusion

Based on the above, it can be stated that the current legislation (Directive 92/118/EC) does not appear the most appropriate, because:

1. the five specific viruses given (foot-and-mouth disease, bluetongue, Rift valley fever, rinderpest and vesicular stomatitis) only encompass RNA viruses;
2. no indication is given about the amount of virus that should be inactivated by the full treatments; and
3. viral infections often run a subclinical course, and as a consequence supervision by the competent authorities in approved slaughterhouses does not guarantee absence of viruses from blood and blood products.

7. Recommendations

7.1 Inactivation

To minimise the risk of viruses being introduced in an animal or animal population, directly or indirectly by the use of blood products, it would be advisable to use representatives of various virus families for the validation of methods aimed at inactivating virus infectivity in blood and blood products.

Instead of using only the five viruses mentioned above, it appears to be more appropriate to expand the panel of viruses and to use members of various virus families. The following categories are proposed: DNA or RNA, single stranded or double stranded, and naked or enveloped. This would result in eight viruses to be used for validation of methods directed at inactivation of viruses. This procedure would also be the best safeguard as to yet unknown viruses. The following bovine viruses can be proposed for this purpose: parvovirus, adenovirus, bovine herpesvirus-1 (=infectious bovine rhinotracheitis virus), foot-and-mouth disease virus, bluetongue virus, bovine viral diarrhoea virus. (There are no single-stranded, enveloped DNA or double-stranded, enveloped RNA viruses that infect bovines).

7.2 Verification of inactivation

The use of virus inactivation treatments, for which inactivation in verifiable is recommended. An example of such a treatment is acidification, in which the enzyme lactate dehydrogenase can be used as a marker.

In many circumstances, it is necessary to check a serum batch after treatment for residual infectious virus in young animals (detecting virus replication and antibody formation) rather than in cell culture, because the former method may be more sensitive. In addition, it is easier to test large volumes in animals than in cell culture. However, sensitivity of virus detection may vary per virus. For instance, for detection of bluetongue virus the most sensitive would be sheep inoculation, whereas for foot-and-mouth disease virus detection cell culture inoculation appears to be more sensitive than animal inoculation.

The Committee for Veterinary Medicinal Products (CVMP) recommends that the treatments reduce the infectious virus titres in bovine blood and blood products by at least a factor of 10^6, for the use of foetal bovine serum for the production of live and killed vaccines (CVMP, 1993). These guidelines may not be totally effective in the case of very high virus titres, which can occur in infections such as bluetongue.

Inactivation procedures that produce a linear inactivation curve are recommended, because inactivation to an acceptable level can then be best calculated (Thomas et al., 1981). A nonlinear inactivation may make it impossible to extrapolate accurately curves beyond the virus detection limit. Ideally, extrapolation of the linear inactivation curve should indicate that there is less than 1 infectious unit per 10,000 litres of serum at the end of inactivation.

Following the inactivation treatments the properties of the serum for promoting cell growth should not be strongly reduced.

7.3. Future considerations

1. To select countries where the bovine blood can be collected, e.g. BVDV free countries, or to select the source of animals carefully.
2. To investigate inactivation methods according to the Committee for Proprietary Medical Products (CPMP) guideline requirements (CPMP/BWP/268/95) and to publish the data (Willkommen et al., 1999). The CPMP guideline also includes:
    a) testing the capacity of the production processes to remove or inactivate viruses,
    b) testing the product at appropriate stages of production for freedom from detectable viruses.
3. To revalidate and optimise the assay systems and their sensitivity (detection limit) for detecting infectious viruses.
4. It may be beneficial to develop and apply sensitive systems for detecting genomes of possible contaminants.
5. Further research to develop cell culture supplement replacements for foetal calf serum and bovine serum products.

8. References

Anonymous, 1986. BVD virus, contamination of fetal bovine serum and infection of cell cultures. Art to Science, 5, 31.

CPMP/BWP/268/95, 1996. Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses.

CVMP. 1994 The rules governing medicinal products in the European Community, Vol VII, Guidelines for the testing of veterinary medicinal products, July 1994, .

Daley JP, Danner DJ, Weppner DJ and Plavsic MZ, 1998. Virus inactivation by gamma irradiation of fetal bovine serum. Focus, 20, 86-88.

Hanson G and Foster L, 1997. Viral safety in serum for cell culture use. Art to Science, 16, 1-7.

Houe H, 1999. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. Veterinary Microbiology; 64, 89-107, 1999.

House C, House JA, and Yedloutschnig RJ, 1990. Inactivation of viral agents in bovine serum by gamma irradiation. Canadian Journal of Microbiology; 36, 737-740.

Straver PJ, Journee DLH and Binkhorst GJ, 1983. Neurological disorders, virus persistence and hypomyelination in calves due to intrauterine infections with bovine virus diarrhoea virus. II. Virology and epizootiology. Veterinary Quarterly; 5, 156-164.

Sullivan R, Fassolitis AC, Larkin EP, Read jr RB, and Peeler JT, 1971. Inactivation of thirty viruses by gamma radiation. Applied Microbiology; 22, 61-65.

Thomas FC, Davies AG, Dulac GC, Willis NG, Papp-Vid G, and Girard A, 1981. Gamma ray inactivation of some animal viruses. Canadian Journal of Comparative Medicine; 45, 397-399.

Tully JG, 1980. Cloning and filtration techniques for Mycoplasmas. In: Methods in Mycoplasmalogy, vol 1. Pp 173-177.

Willkommen H, Scheiblauer H, and Löwer J, 1999. Serum and serum substitutes: virus safety by inactivation or removal. Developments in Biological Standardisation, 99, 131-138.