



97/1502  
5 May 2000

## PROPOSED QUARANTINE REQUIREMENTS FOR THE IMPORTATION OF CERVINE EMBRYOS FROM POLAND

### 1 GENERAL

- 1.1 Only *in vivo* fertilised embryos are eligible for export to Australia. Ova and embryos fertilised *in vitro* are not eligible for export.
- 1.2 Before the embryos can be exported, a valid *Permit to Import Quarantine Material* must be obtained from the Australian Quarantine and Inspection Service (AQIS) office in the State of import and accompany each consignment of cervine embryos. The exporter must ship the cervine embryos to the Australian importer, care of AQIS in the State of import.
- 1.3 The Animal Health Certificate must:
- accompany each consignment of cervine embryos (copies not acceptable);
  - conform to the format shown in Attachment 1 and, under **V. Sanitary Information**, contain the certifications specified in Section 2 of these requirements;
  - record dates of sampling for tests, the isolation period and the embryo collection dates;
  - be signed by the *certifying veterinarians*, that is,
    - the *Team Veterinarian* who is a veterinarian specifically approved by the veterinary administration of Poland to supervise the Collection Team, in accordance with Office International des Épizooties (OIE) International Animal Health Code (*Code*) Appendix 4.2.3.9. (Attachment 2); and
    - the *Official Veterinarian* who is a civil service veterinarian or a specially appointed veterinarian as authorised by the veterinary administration of Poland;
  - be in English, and a language understood by the *certifying veterinarians*, and
  - be stamped on each page with an Official stamp.
- 1.4 Permission to import cervine semen or embryos must also be obtained from Environment Australia to meet the requirements of the *Wildlife Protection (Regulation of Exports and Imports) Act 1982*. Further information may be obtained from:
- |                       |  |
|-----------------------|--|
| The Director          | Ph: 02 6274 2291   |
| Wildlife Protection   | Fax 02 6274 1921   |
| Environment Australia | email wps@ea.gov.au  |
| GPO Box 787           | website: <a href="http://www.biodiversity.environment.gov.au/plants/wildlife/intro.htm">http://www.biodiversity.environment.gov.au/plants/wildlife/intro.htm</a> |
| Canberra ACT 2601     |  |
- 1.5 Section 2 sets out the minimum requirements for importation into Australia. Various zones in Australia may differ in animal health status and State/Territory veterinary authorities may require

testing or certification additional to these requirements before the semen may enter a particular zone or move from one zone to another within Australia.

1.6 Either the *Team Veterinarian* or the *Official Veterinarian* must supervise:

- all sample collections for diagnostic tests,
- all collections of embryos, and
- all servicing of storage containers prior to export.

The program may be subject to direct audit by AQIS at some stage during the collection period.

1.7 Any requests for dispensation from these requirements must be submitted through the veterinary administration of Poland. Such applications must include the reasons and contain all information necessary for the application to be evaluated. AQIS will only consider requests for dispensation received through the veterinary administration of Poland with their recommendation. Dispensations will be issued in exceptional circumstances by AQIS when it can be demonstrated that the quarantine security of the consignment has not been compromised.

1.8 All references to the Office International des Épizooties International Health Code (OIE *Code*) refer to the 8<sup>th</sup> edition, 1999.

1.9 Conditions of importation may be varied or reviewed at any time at the discretion of AQIS.

## 2. CERTIFICATION

The Animal Health Certificate must attest, under **V. Sanitary information**, that:

2.1 Each donor (male and female) was kept:

- free from any quarantine restriction for 60 days prior to starting the 30-days pre-collection centre residency period;
- for 3 months in a country or zone recognised by the OIE as a foot and mouth disease (FMD) free country where vaccination is not practised (Article 2.1.1.2.), and
- in a country or zone which meets the OIE *Code* Article requirements for freedom from the following diseases:
  - rinderpest [free country] (Article 2.1.4.2.);
  - Rift Valley fever (Article 2.1.8.2.), and
  - vesicular stomatitis (Article 2.1.2.2.).

2.2 Bluetongue, epizootic haemorrhagic disease of deer, louping ill and chronic wasting disease of deer (CWD) have not been reported in Poland.

2.3 There was no report of Borna disease in horses or ruminants in the District (Wojwodship) of Poland where the deer was kept during the 12 months prior to collection.

2.4 Each donor (male and female) was free from clinical signs of bovine tuberculosis and came from herds free from bovine tuberculosis in which:

- all animals over 8 months of age had reacted negatively to two official intradermal tuberculin tests, the first being six months after the first whole herd negative test, the second test three to six months later; and

- all animals in the herds had tested negative to an annual intradermal tuberculin test.

- 2.5 Each donor (male and female) came from herds free from bovine brucellosis:  
either  
in a District (Wojwodship) of Poland where all bovine herds were officially free from bovine brucellosis and where there was no report of bovine brucellosis for the previous five years,  
or  
in which there was no report of clinical brucellosis for at least six months and in which all animals in the herds were subjected to official complement fixation tests (CFT) for bovine brucellosis on two occasions, with an interval of 3 to 12 months between each test.
- 2.6 All donors were of the same health status and were kept isolated from other non-cervine species for 30 days prior to, and during, the collection period on the centre used for the collection of embryos for this consignment.
- 2.7 During the pre-collection period each donor (male and female) gave a negative result to the following tests:
- a mid-cervical intradermal tuberculin test;  
*(Note: Tests for bovine tuberculosis must be carried out at least 90 days after any previous tuberculin test in the manner approved by the Veterinary Administration of Poland);*
  - a CFT for bovine brucellosis, and
  - a serum neutralisation test (SNT) for infectious bovine rhinotracheitis.
- 2.8 The health status of each semen donor was equivalent to those prescribed for the female donors and the embryos were produced by observed natural service using identified males.
- 2.9 The embryos in this consignment were fertilised *in vivo*; were not subjected to micromanipulation involving breaching of the zona pellucida; and all had intact zona pellucida at the time of storage.
- 2.10 All embryos in this consignment were collected, processed and stored in accordance with the procedures detailed in OIE *Code* Appendix 4.2.3.9. The embryos were washed 10 times in accordance with the International Embryo Transfer Society (IETS) Manual recommendations.  
*(Note: There is no requirement that an enzymatic wash be included in the washing process.)*
- 2.11 The *Team Veterinarian*:
- supervised the placing of embryos in this consignment in fresh liquid nitrogen in a new or properly disinfected container for export; and
  - ensured that the container contained only other semen or embryos collected for export to Australia.
- 2.12 The *Official Veterinarian*:
- verified the identification of the embryos with the identification details of the donor(s); and
  - sealed the container and recorded the number or mark on the seal on the certificate prior to export.

### 3 IMPORTER'S/AGENT'S RESPONSIBILITIES

- 3.1 It is the responsibility of the importer or importer's agent to arrange for the provision of any health certification or testing additional to that required by AQIS (eg for inherited diseases or genetic defects, or as required by State/Territory veterinary authorities).
- 3.2 The importer or agent must nominate a person who can be contacted by AQIS officers and who will be responsible for ensuring that all import requirements are met.
- 3.3 The Australian Government will charge the importer for services provided. The Australian Government will not compensate the importer or agent for any losses incurred while the semen intended for importation is under AQIS control.

### 4 POST ARRIVAL

- 4.1 AQIS will hold the consignment until a Quarantine Veterinary Officer (QVO) or a Quarantine Officer, under the direct supervision of a QVO, has audited the contents of the shipping container.
- 4.2 In the event of a transport container arriving in Australia without the correct certification, with the seals on the transport containers broken or in any other way not having met these requirements, AQIS may place the container and its entire contents in quarantine, return it to the country of origin or destroy it without recompense.

SARAH KAHN  
Assistant Director  
Animal Quarantine Policy Branch

ANIMAL HEALTH CERTIFICATE

Import Permit Number: .....  
Species and Category: CERVINE EMBRYOS  
Importing Country: AUSTRALIA.  
Exporting Country: POLAND

**I Information concerning each donor**

- a) Does:
  - Breed:
  - Herd book number:
  - Identification:
  - Herd of origin:
- b) Stags
  - Breed:
  - Herd book number:
  - Identification:
  - Herd of origin:

**II Information concerning embryos and semen from each donor**

<i>Embryos</i>	<i>Semen</i>
Dates of collection:	Date of collection:
Number of embryos:	Number of straws:
Number of straws:	Straw identification:
Straw identification:	

**III Origin of the embryos**

Exporter Name :  
 Address :  
 Registered name of the approved embryo collection team :  
 Name and address of premises at which embryos collected :

**IV Destination of the embryos**

Consignee - Name: Chief Quarantine Officer (Animals)  
 Address  
 Nature and identification of means of transport:

**V Sanitary information**

The undersigned Embryo Collection *Team Veterinarian* and the undersigned *Official Veterinarian* certify in respect of the donor animals described in part I of this certificate, and in respect of the cervine embryos described in part 2 of this certificate, that:  
 (Certification as detailed in Section 2 of this document)

Signature:.....Date:.....  
 (Embryo Collection Team Veterinarian)

Signature:.....Date:.....  
 (Official Veterinarian)

*Note: Official Stamp must be endorsed on all pages.*

APPENDIX 4.2.3.9.

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CERVID EMBRYOS/OVA

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**A. AIMS OF CONTROL**

The purpose of official sanitary control of fresh or frozen embryos/ova intended for movement internationally is to ensure that specific pathogenic organisms which could be associated with embryos/ova are controlled, and infection in recipient animals and progeny is avoided.

The disease situation between *exporting* and *importing countries* may be similar or dissimilar, and national prophylactic programmes can vary widely, as can vaccination and testing requirements. Thus, exporting and importing countries may have different conditions for the approval of embryo collection teams and associated processing laboratories. For these and other reasons, this Appendix recommends only the main sanitary conditions under which embryos/ova may be collected, processed and transported. The recommendations apply principally to embryos from animals continuously resident in national domestic or ranched cervid populations and therefore may be found to be unsuitable for those from cervids in feral or other circumstances related to biodiversity or germplasm conservancy efforts.

**B. GENERAL CONDITIONS**

The *Veterinary Administration* should ensure that the general conditions relating to animal health set out in the following paragraphs are followed for the international movement of embryos/ova.

**1. Embryo collection team**

Embryo collection team means a group of competent technicians, including at least one veterinarian, to perform the collection, processing and storage of embryos. The following conditions should apply:

- a) The team should be supervised by a team veterinarian.
- b) The team veterinarian is responsible for all team operations, which include sanitary handling of donors, disinfection and hygienic procedures.
- c) The team veterinarian should be specifically approved for this purpose by an *Official Veterinarian*.
- d) Team personnel should be adequately trained in the techniques and principles of disease control. High standards of hygiene should be practised to preclude the introduction of infection.
- e) The collection team must function in adequate facilities and possess proper equipment for:
  - . collecting embryos;
  - . processing and treatment of embryos at a permanent site or mobile laboratory;
  - . storing embryos.
- f) As these facilities are not necessarily at the same location, the collection team must keep a record of its activities which must be maintained for inspection by the approving authority.
- g) The collection team should be subjected to regular inspection to ensure compliance with sanitary collection, processing and storage of embryos.
- h) The collection team must not operate in an *infected zone* unless the disease in question has been listed by the International Embryo Transfer Society (IETS) as one on which sufficient research has been done to demonstrate that the risk of its transmission by embryo transfer is negligible\*.

## **2. Processing laboratories**

The processing laboratory used by the collection team may be mobile or permanent. It is a facility in which embryos/ova are recovered from collection media, examined and subjected to any required treatments such as washing before freezing, storage and quarantine, pending results of health control tests.

A permanent laboratory may be part of a specifically designed collection and processing unit, or a suitably adapted part of an existing building on the premises where donor animals are kept. In either case, the laboratory should be physically separated from animals. Both mobile and permanent laboratories should have a clear separation between dirty areas (animal handling) and the clean processing area.

Additionally:

- a) The laboratory should be under the direct supervision of the team veterinarian and be regularly inspected by an Official Veterinarian.
- b) While embryos/ova for export are being handled prior to their storage in ampoules/straws, no embryos/ova of a lesser health status should be processed.
- c) The laboratory should be protected against rodents and insects.
- d) The processing laboratory should be constructed with materials which permit its effective cleansing and disinfection. This should be done following each occasion on which embryos are processed.
- e) The laboratory must not be situated in an infected zone unless the disease in question has been listed by the IETS as one for which the risk of transmission by embryo transfer has been shown to be negligible\*.

## **3. Donor animals**

- a) At the time of collection, donor animals should be clinically inspected by the team veterinarian and confirmed to be free of clinical evidence of contagious and infectious diseases transmissible to cervids.
- b) The herd of origin meet the requirements provided for in the Articles dealing with embryos/ova in the *Code* Chapters on foot and mouth disease, rinderpest, vesicular stomatitis (under study), bluetongue and peste des petits ruminants.
- c) The Veterinary Administration should have knowledge of, and authority over, the herd of origin of the donor animals.
- d) The donor animals should not have been imported from another country during the previous 60 days and should have been in the herd of origin for at least 30 days.

## **4. Testing of donor animals and embryos/ova**

Embryo transfer is acknowledged as a very low risk method for moving animal genetic material. Irrespective of animal species, there are two main approaches for providing the desired levels of protection that imported embryos/ova are free of pathogenic organisms: the so-called "traditional" method and the embryo processing method.

The traditional approach is based on testing of the donor animal before, during or after embryo collection depending on the incubation period and pathogenesis of the disease of concern. Analysis of the sera

enables the health status of the donor to be determined. This has its most significant application in the case of frozen embryos where clinical examination and determination of the donor status can be verified retrospectively.

The alternative methodology involves embryo processing, such as washing, which are now widely used for the embryos of other species (e.g. cattle), based on documented research involving those species which has determined that zona pellucida intact embryos/ova if processed in accordance with the Manual of the International Embryo Transfer Society have a high level of freedom from certain pathogens. While washing is also recommended for cervid embryos, its efficiency for pathogen removal is unknown due to a lack of specific research.

It is necessary, therefore, to rely more on traditional approaches to ensure that cervid embryos/ova are free of pathogenic organisms. These approaches include clinical checking and testing of donor animals at or just before embryo collection and then re-checking and/or re-testing afterwards, taking account of normal incubation periods of the diseases of concern. In the interval the embryos may be stored frozen, in liquid nitrogen, in the exporting country.

Semen used to inseminate donor animals artificially or fertilise ova should meet health requirements and standards as established bilaterally between the respective Veterinary Administrations.

When frozen semen collected from bucks no longer living is used to inseminate donor animals, and when the health status of the buck concerning a particular infectious disease or diseases was not known at the time of collection, additional tests may be required of the inseminated donor female after embryo collection, to verify that these infectious diseases were not transmitted. An alternative may be to subject the semen to testing.

Where natural service or fresh semen is used, sires should meet the same health requirements as donor females.

## **5. Collection and storage of embryos/ova**

### **a) Media**

Any biological product of animal origin used in the media and solutions for collection, processing or storage of embryos/ova should be free of pathogenic micro-organisms. Media and solutions should be sterilised by approved methods according to the IETS Manual\*\*, and handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to collection, processing and storage media as recommended in the IETS Manual.

### **b) Equipment**

All equipment used to collect, handle, wash, freeze and store embryos/ova should be sterilised prior to use in accordance with recommendations in the IETS Manual.

## **6. Optional tests\*\*\* and treatment**

a) The examination of the collection and washing fluids, and, if available, of unfertilized ova and degenerated embryos/ova, can be requested by an importing country. Tests to confirm the absence of pathogenic organisms, or to assess whether the degree of quality control of the collection team is of an acceptable standard, may be carried out on any of the following samples:

### **i) Collection fluids**

Collection fluids should be placed in a sterile, closed container and, if there is a large amount, it should be allowed to stand undisturbed for approximately 1 hour. Excess supernatant fluid should then be removed and the bottom 10-20 ml, along with accumulated debris, decanted into a sterile bottle for testing. If a filter is used in the collection of embryos/ova, then any debris that is retained on the filter must be rinsed

into the retained fluid.

ii) Washing fluids

The last four washes of the embryos/ova (washes 7, 8, 9 and 10 - IETS Manual) should be pooled for testing.

iii) Samples

The samples referred to above should be stored at 4°C and tested within 24 hours. If this is not possible, then samples should be stored frozen at -70°C or lower.

- b) Treatment of the viable embryos/ova by washing 10 times may be requested by the importing country and, if so, should be carried out according to the IETS Manual. Only embryos/ova from one donor should be processed simultaneously. It should be noted that the effect of enzymatic treatment on the viability of cervid embryos/ova and its efficacy against specified pathogenic organisms of Cervidae has not yet been determined.

## **7. Storage, quarantine and transport**

- a) The embryos/ova should be stored for transport in sealed sterile ampoules, vials or straws, under strict hygienic conditions at a storage place, approved by the Veterinary Administration of the *exporting country*, where no risk of contamination can occur.
- b) Only embryos/ova from the same donor should be stored together in the same ampoule, vial or straw.
- c) Embryos/ova should be frozen and stored in fresh liquid nitrogen.
- d) Liquid nitrogen containers should be sealed prior to shipment from the exporting country and should be labelled according to the IETS Manual.
- e) Embryos/ova must not be exported until the appropriate health certification documents are completed.

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\* At the present time, there are no diseases of cervids presently included in the four categories established by the Research Subcommittee of the International Embryo Transfer Society and published in the *Rev. sci. tech. Off. Int. Epiz.* (1992), **11** (3), 937-938.

\*\* Manual of the International Embryo Transfer Society (1997), 309 West Clark Street, Champaign, IL 61820, USA.

\*\*\* If the samples mentioned in paragraph B.6 of this Appendix are to be tested for pathogenic agents, then the microbiological techniques in current use for those agents would be appropriate.