Report of the Scientific Committee on Animal Health and Animal Welfare on the Suggested Protocol for the Importation of Live Animals from Bluetongue Virus (BTV) and Epizootic Haemorrhagic Disease Virus (EHDV) Endemic Areas adopted 21 October 1998

Request for an Opinion

The Scientific Committee has been requested to propose a protocol for the importation of live animals from BTV and EHDV endemic areas.

Bluetongue (BT)

Bluetongue (BT) is an insect-borne infection of ruminants, caused by an orbivirus, with at least 24 known serotypes. The disease is endemic in areas of the Americas, Africa, Eastern Europe, Australia, and Asia where the biological vectors are active throughout the year. It is an OIE list A disease. The specific suggestions mentioned in this paper only cover BTV and EHDV infections. Other arthropod borne virus infections of ruminants, such as for instance, Rift Valley Fever, should be considered separately.

Domestic and wild ruminants can be infected by BT virus, but severe clinical disease is usually seen only in sheep and in some wild ruminants (deer). The severity of clinical signs depends on the virus strain and the species and breed of animal affected. Clinical signs can be very variable, ranging from mild to severe and death. The severity of clinical signs depends on the virus strain as well as the species and breed of animal affected. The clinical signs are described in the OIE Manual of Standards for Diagnostic Tests and Vaccines. The effects on animal welfare vary with the severity of the disease. Subclinical infections are very common in all ruminant species in infected areas.

Transmission takes place via certain species of *Culicoides* biting midges which are biological vectors. Transmission is only via bite. Unlike some insect borne viruses, horizontal or vertical transmission of BTV among the vector is not known to occur (Barratt-Boyes and MacLachlan 1995, Verwoerd and Erasmus, 1994). In the Europe, Africa and the Middle East *C. imicola* is considered as the most important vector, in North America the major vector is *C. variipennis* and in Central and South America it is probably *C. insignis*. In Australia the major vectors are *C. wadai*, *C. brevitarsis*, *C. fulvus* and *C. actoni*. Other species of *Culicoides* have been shown to be less efficient vectors. The efficiency of vector species of *Culicoides* in transmitting BTV varies, depending on the virus strain and the local sub-population of the insect involved.

Viraemia develops after about 3 days and usually peaks at around 10^4 -10^5 ID_{50}/ml at 10 to 15 days after infection. Viraemia may last for up to 54 days in sheep and goats and for up to 100 days in cattle, though it is usually much shorter in all 3 species. In infected animals virus is present in the blood, attached to red blood cells and in such a position is protected from the effects of antibody (Koumbati et al. in press, Barratt-Boyes and MacLachlan 1995, Verwoerd and Erasmus 1994, Katz et al. 1994).

Infected animals develop detectable levels of antibody within four weeks of infection but in many cases antibodies may be detected as early as seven days, which means that a period of concurrent viraemia and antibody may exist for a significant time.

Detectable antibodies persist for longer than the period of viraemia. There is no reliable scientific evidence for immunotolerance occurring with BTV.

For the detection of virus, sheep inoculation, intravenous inoculation of chick embryos and cell cultures are used, in decreasing order of sensitivity. In addition PCR techniques have been developed which are giving promising results.

For the detection of group-specific antibody (all serotypes) the agar gel immunodiffusion (AGID) test and ELISA are

used (OIE Manual of Standards for Diagnostic Tests and Vaccines, 1996). With the AGID, cross reactions with EHDV antibodies are common. A blocking (or competitive) ELISA, (Anderson, 1984), using a monoclonal antibody is highly reliable, because of greater sensitivity and specificity than the AGID (no cross reactions with EHDV antibodies). For the detection of type-specific antibodies virus neutralisation tests are used.

The adoption of International standard reference sera or the carrying out of interlaboratory comparisons is recommended in order to standardise laboratory results.

Epizootic Haemorrhagic disease (EHD)

EHD is an insect-borne virus disease of ruminants and as regards the aspects relevant to this report, is similar to BT.

EHDVs are a group of related and widely distributed orbiviruses, with at least eight serotypes.

Domestic and wild ruminants can be infected, although clinical disease was originally described in deer. Subclinical infections are common in infected areas. EHDV-1 and/or EHDV-2 which have been shown to cause lesions in some species of domestic and wild ruminants are known to occur in America, Africa, Australia and Japan.

For the detection of virus the methods recommended for BTV are used except that cell culture is more sensitive than intravenous inoculation of embryonated hens eggs. For the detection of antibody, a competitive ELISA (Afshar et al., 1997, Thevasagayam et al, 1995, 1996) or the AGID test is used. Certain AGID tests have been shown to lack complete group specificity and cross reactions with BTV antibodies may also occur. Therefore prior to use, any AGID test selected must be proven able to detect all known serotypes of EHDV and positive reactions must be confirmed by virus neutralisation tests or by a competitive ELISA

Endemic, epidemic and free areas

The northern and southern boundaries of BTV and EHDV infection around the world are determined by the presence of vector species of *Culicoides,* the distribution of which is controlled by climate. Areas where adult vectors occur throughout the year are actual or potential BTV and EHDV endemic areas, areas where adult vectors occur only seasonally are potential BTV and EHDV epidemic areas.

A distinct borderline between endemic, epidemic and free areas cannot always be drawn with certainty due to the movement of known vectors in response to climatic fluctuations and other environmental factors, and to the existence of less efficient potential vectors.

Recommendations

The Scientific Committee for Animal Health and Animal Welfare proposes the following protocol, based on testing and quarantine of individual animals, as a useful tool in connection with EU importation of live animals.

The final recommendations are based on thorough consideration of published knowledge on BT and EHD, on previous suggestions for protocols, on the current Office International des Epizooties (OIE) guidelines (Code and Manual), and on an assessment of the risks involved in importation of live animals.

Suggested protocol:

Live ruminants can be imported from bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) endemic or epidemic areas under the following conditions:

Seronegative Animals

These animals should be kept in quarantine under vector-free conditions [1] for at least 40 days [2] , be sampled and tested

at the beginning of the quarantine period and at least 28 days later and have been shown to be negative for antibodies against BTV and EHDV [3]. If a positive animal is identified, it should be removed and all other animals re-quarantined and sampled and tested 28 days later with negative result.

Seropositive Animals

If seropositive, virus negative, animals are to be considered for importation into the EU the following protocol may be useful. However, it should be noted, that such seropositive animals, may later cause potential problems in establishing convincing evidence for absence of infection by routine serosurveys or during re-export. Such animals may represent an additional risk of introducing the infection.

However, a suitable protocol could provide an acceptable safeguard and is as follows:

The animal is kept in quarantine under vector-free conditions [1] until completion of the protocol (with a minimum period of 40 days [2].) During this period at least 40 ml of blood shall be collected from the animal on each of two separate occasions at least 7 days apart, the first collection being taken in the first week of quarantine. The blood samples shall be heparinised and shall be kept under sterile conditions at 4 ^{o}C for a maximum of 10 days before inoculation into sheep for the purposes of virus detection. The two samples from the animal are pooled and half of the total volume (40 ml) is inoculated subcutaneously into each of two sheep [4] (previously shown to be negative for antibodies against BTV and EHDV [3]). Both sheep must remain BTV and EHDV seronegative when sampled and tested at 28 days, with a second sample taken and tested at least 35 days after the inoculation.

Notes

Note 1 Vector-free conditions

Vector-free conditions are, in this situation, defined as either quarantine facilities equipped to exclude vectors, or quarantine in a vector-free area, or during a vector-free season. In each case, the animals designated for export must be treated with a suitable insect repellent before leaving the farm of origin and with an effective insecticide on arrival at the quarantine facility. Vector-free conditions should be established for transport of the animals from the quarantine facility to the final destination for entry into the EU. Quarantine facilities should be all-in all-out. A vector-free season, may tentatively be defined as a period starting with killing frost (below minus 3°C for several hours) followed by at least 10 consecutive days with frost outside the quarantine facility. The period should end when 10 out of 20 consecutive days reach temperatures above 10°C.

Note 2 Forty Days Quarantine Period

The decision for a period of 40 days for quarantine is based on published data on the level and duration of viraemia during BTV infection (Barratt-Boyes and MacLachlan, 1995). Based on this knowledge, the level of viraemia will decrease by at least 2-3 logs during a 40 day period and thus substantially reduce the risk of introducing the infection. Thus, the period itself is an extra safeguard, should the serological or virological examination of the animals fail to identify an infected animal (see note 3).

Note 3 Sero-diagnostic methods

The sero-diagnostic methods used should be competitive ELISA methods or AGID tests as specified in the "OIE Manual of Standards for Diagnostic Tests and Vaccines" using the appropriate BTV and EHDV group specific antigens. The competitive ELISA is generally regarded as being more sensitive than the AGID test and therefore its use is recommended. To increase confidence, the committee suggests having 2 tests performed, one at the beginning and the second towards the end of the quarantine (at least 28 days apart), in order to minimise the possibility of false negatives. The combined effect of two negative tests and a quarantine period of at least 40 days should result in an

acceptable level of risk.

The adoption of International standard reference sera is strongly recommended. The carrying out of interlaboratory comparisons is regarded as essential in order to standardise laboratory tests .

Note 4 New Techniques

The Committee notes that PCR techniques have been developed which are giving promising results and which would eliminate the need for animal inoculation (Katz et al., 1993; MacLachlan et al., 1994). The Committee recommends that the use of the PCR be kept under review with a view to the replacement of current animal testing methods. To this end, comparative data on both test methods should be obtained and evaluated.

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