

Terrestrial Animal Health Standards  
Commission Report

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CHAPTER 12.1.

**AFRICAN HORSE SICKNESS**

Article 12.1.1.

For the purposes of the Terrestrial Code, the infective period for African horse sickness virus (AHSV) shall be 40 days for domestic horses. Although critical information is lacking for some species, this Chapter applies to all equidae.

All countries or zones neighbouring, or considered to be at risk from, a country or zone not having free status should determine their AHSV status from an ongoing surveillance programme. Throughout the Chapter, surveillance is in all cases understood as being conducted as described in Chapter 1.4.

Standards for diagnostic tests and vaccines are described in the Terrestrial Manual.

Article 12.1.2.

**AHSV free country or zone**

1. A country or zone may be considered free from AHSV when African horse sickness (AHS) is notifiable in the whole country, systematic vaccination is prohibited, importation of equidae and their semen and oocytes or embryos are carried out in accordance with this Chapter, and either:
  - a) historical freedom as described in Chapter 1.4. has demonstrated no evidence of AHSV in the country or zone; or
  - b) the country or zone has not reported any case of AHS for at least 2 years and is not adjacent to a country or zone not having a free status; or
  - c) a surveillance programme has demonstrated no evidence of AHSV in the country or zone for at least 12 months and includes a complete season of vector activity; or
  - d) the country or zone has not reported any case of AHS for at least 40 days and a surveillance programme has demonstrated no evidence of Culicoides likely to be competent AHSV vectors for at least 2 years in the country or zone.
2. An AHSV free country or zone will not lose its free status through the importation of vaccinated or seropositive equidae and their semen, oocytes or embryos from infected countries or infected zones, provided these imports are carried out in accordance with this Chapter.

## Article 12.1.3.

**AHSV seasonally free zone**

1. An AHSV seasonally free zone is a part of an infected country or an infected zone ~~for~~ in which for part of a year, ongoing surveillance and *monitoring consistently* demonstrated ~~no~~ neither evidence of AHSV transmission ~~and~~ nor the evidence of the presence of adult Culicoides likely to be competent AHSV vectors.
2. For the application of Articles 12.1.6., 12.1.8. and 12.1.9., the seasonally free period is:
  - a) taken to commence the day following the last evidence of AHSV transmission and of the cessation of activity of adult Culicoides likely to be competent AHSV vectors as demonstrated by an ongoing surveillance programme, and
  - b) taken to conclude either:
    - i) at least 40 days before the earliest date that historical data show AHSV activity has recommenced; or
    - ii) immediately when current climatic data or data from a surveillance and monitoring programme indicate an earlier resurgence of activity of adult Culicoides likely to be competent AHSV vectors.
3. An AHSV seasonally free zone will not lose its free status through the importation of vaccinated or seropositive equidae and their semen, oocytes or embryos from infected countries or infected zones, provided these imports are carried out in accordance with this Chapter.

## Article 12.1.4.

**AHSV infected country or zone**

An AHSV infected country or infected zone is one in which the conditions of Article 12.1.2. or Article 12.1.3. do not apply.

## Article 12.1.5.

**Recommendations for importation from AHSV free countries that are neither neighbouring nor considered to be at risk from an AHSV infected country or infected zone**for equidae

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the animals:

1. showed no clinical sign of AHS on the day of shipment;
2. have not been vaccinated against AHS within the last 40 days;
3. were kept in an AHSV free country since birth or for at least 40 days prior to shipment;

4. either:
  - a) did not transit through an infected country or infected zone; or
  - b) were protected from attacks by Culicoides at all times when transiting through an infected country or infected zone.

Article 12.1.6.

**Recommendations for importation from AHSV free countries or free zones or from AHSV seasonally free zones (during the seasonally free period) that are neighbouring or are considered to be at risk from an AHSV infected country or infected zone**

for equidae

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the animals:

1. showed no clinical signs of AHS on the day of shipment;
2. have not been vaccinated against AHS within the last 40 days;
3. were kept in an AHSV free country, free zone or seasonally free zone during the seasonally free period since birth or for at least 40 days prior to shipment; or
4. in a country or zone considered to be at risk, were held in quarantine for at least 40 days prior to shipment and protected at all times from attacks by Culicoides; and
  - a) a serological test according to the *Terrestrial Manual* to detect antibodies to the AHSV group, was carried out with a negative result on a blood sample collected at least 28 days after introduction into the quarantine station; or
  - b) serological tests according to the *Terrestrial Manual* to detect antibodies against AHSV were carried out with no significant increase in antibody titre on blood samples collected on two occasions, with an interval of not less than 21 days, the first sample being collected at least 7 days after introduction into the quarantine station; or
  - c) agent identification tests according to the *Terrestrial Manual* were carried out with negative results on blood samples collected on two occasions with an interval of not less than 14 days between collection, the first sample being collected at least 7 days after introduction into the quarantine station;
5. were protected from attacks by Culicoides at all times during transportation (including to and at the place of shipment).

## Article 12.1.7.

**Recommendations for importation from AHSV infected countries or zones**for equidae

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the animals:

1. showed no clinical sign of AHS on the day of shipment;
2. have not been vaccinated against AHS within the last 40 days;
3. were held continuously during the quarantine period of at least 40 days, in a vector-proof quarantine station and protected at all times from attacks by *Culicoides*; and
  - a) a serological test according to the *Terrestrial Manual* to detect antibodies to the AHSV group, was carried out with a negative result on a blood sample collected at least 28 days after introduction into the quarantine station; or
  - b) serological tests according to the *Terrestrial Manual* to detect antibodies against AHSV were carried out with no significant increase in antibody titre on blood samples collected on two occasions, with an interval of not less than 21 days, the first sample being collected at least 7 days after introduction into the quarantine station; or
  - c) agent identification tests according to the *Terrestrial Manual* were carried out with negative results on blood samples collected on two occasions with an interval of not less than 14 days between collection, the first sample being collected at least 7 days after introduction into the quarantine station;
4. protected from attacks by *Culicoides* at all times during transportation (including during transportation to and at the place of shipment).

## Article 12.1.8.

**Recommendations for the importation of equid semen**

*Veterinary Authorities* of *importing countries* should require the presentation of an *international veterinary certificate* attesting that the donor animals:

1. showed no clinical sign of AHS on the day of collection of the semen and for the following 40 days;
2. had not been ~~vaccinated~~ immunised against AHS with a live attenuated vaccine within 40 days prior to the day of collection;
3. were either:

- a) kept in an AHSV free country or free zone or from an AHSV seasonally free zone (during the seasonally free period) for at least 40 days before commencement of, and during collection of the semen, or
- b) kept in an AHSV free vector-proof artificial insemination centre throughout the collection period, and subjected to either:
  - i) a serological test according to the *Terrestrial Manual* to detect antibody to the AHSV group, carried out with a negative result on a blood sample collected at least 28 days and not more than 90 days after the last collection of semen; or
  - ii) agent identification tests according to the *Terrestrial Manual* carried out with negative results on blood samples collected at commencement and conclusion of, and at least every 7 days, during semen collection for this consignment.

Article 12.1.9.

### **Recommendations for the importation of in vivo derived equid embryos/oocytes**

*Veterinary Authorities of importing countries* should require the presentation of an *international veterinary certificate* attesting that:

1. the donor animals:
  - a) showed no clinical sign of AHS on the day of collection of the embryos/oocytes and for the following 40 days;
  - b) had not been ~~vaccinated~~ immunised against AHS with a live attenuated vaccine within 40 days prior to the day of collection;
  - c) were either:
    - i) kept in an AHSV free country or free zone or from an AHSV seasonally free zone (during the seasonally free period) for at least 40 days before commencement of, and during collection of the embryos/oocytes, or
    - ii) kept in an AHSV free vector-proof collection centre throughout the collection period, and subjected to either:
      - a serological test according to the *Terrestrial Manual* to detect antibody to the AHSV group carried out with a negative result on a blood sample collected at least 28 days and not more than 90 days after the last collection of embryos/oocytes; or
      - agent identification tests according to the *Terrestrial Manual* carried out with negative results on blood samples collected at commencement and conclusion of, and at least every 7 days during embryos/oocytes collection for this consignment;

2. the embryos were collected, processed and stored in conformity with the provisions of Chapter 4.7.;
3. semen used to fertilize the oocytes, complies at least with the requirements in Article 12.1.8.

Article 12.1.10.

### **Protecting animals from Culicoides attack**

When transporting equines through AHSV infected countries or AHSV infected zones, *Veterinary Authorities* should require strategies to protect animals from attacks by Culicoides during transport, taking into account the local ecology of the vector.

Potential *risk management* strategies include a combination of:

1. treating animals with chemical repellents prior to and during transportation, in sanitized vehicles treated with appropriate residual contact insecticide;
2. *loading*, transporting and *unloading* animals at times of low vector activity (i.e. bright sunshine and low temperature);
3. ensuring *vehicles* do not stop en route during dawn or dusk, or overnight, unless the animals are held behind insect proof netting;
4. darkening the interior of the vehicle, for example by covering the roof and/or sides of vehicles with shade cloth;
5. monitoring for vectors at common stopping and offloading points to gain information on seasonal variations;
6. using historical, ongoing and/or AHS modelling information to identify low risk ports and transport routes.

Article 12.1.11.

### **Surveillance: introduction**

Articles 12.1.11. to 12.1.13. define the principles and provides a guide on the surveillance for AHS, complementary to Chapter 1.4., applicable to Members seeking to determine their AHSV status. This may be for the entire country or zone. Guidance for Members seeking free status following an outbreak and for the maintenance of AHS status is also provided.

AHS is a vector-borne infection transmitted by a limited number of species of Culicoides insects. Unlike the related bluetongue virus, AHSV is so far geographically restricted to sub Saharan Africa with periodic excursions into North Africa, southwest Europe, the Middle East and adjacent regions of Asia. An important component of AHSV epidemiology is vectorial capacity which provides a measure of disease risk that incorporates vector competence, abundance, seasonal incidence, biting rates, survival rates and the extrinsic incubation period. However, methods and tools for measuring some of these vector factors remain to be developed, particularly in a field context.

According to this Chapter, a Member demonstrating freedom from AHSV infection for the entire country, or a zone should provide evidence for the existence of an effective surveillance programme. The strategy and design of the surveillance programme will depend on the prevailing epidemiological circumstances and should be planned and implemented according to general conditions and methods described in this Chapter. This requires the support of a laboratory able to undertake identification of AHSV infection through the virus detection and antibody tests described in the Terrestrial Manual.

Susceptible wild equid populations should be included in the surveillance programme.

For the purposes of surveillance, a case refers to an equid infected with AHSV.

The purpose of surveillance is to determine if a country or zone is free from AHSV or if a zone is seasonally free from AHSV. Surveillance deals not only with the occurrence of clinical signs caused by AHSV, but also with evidence of infection with AHSV in the absence of clinical signs.

The following defines the occurrence of AHSV infection:

1. AHSV has been isolated and identified as such from an equid or a product derived from that equid, or
2. viral antigen or viral RNA specific to one or more of the serotypes of AHSV has been identified in samples from one or more equids showing clinical signs consistent with AHS, or epidemiologically linked to a confirmed or suspected case, or giving cause for suspicion of previous association or contact with AHSV, or
3. serological evidence of active infection with AHSV by detection of seroconversion with production of antibodies to structural or nonstructural proteins of AHSV that are not a consequence of vaccination have been identified in one or more equids that either show clinical signs consistent with AHS, or epidemiologically linked to a confirmed or suspected case, or give cause for suspicion of previous association or contact with AHSV.

Article 12.1.12.

#### **Surveillance: general conditions and methods**

1. A surveillance system should be under the responsibility of the Veterinary Authority. In particular the following should be in place:
  - a) a formal and ongoing system for detecting and investigating outbreaks of disease;
  - b) a procedure for the rapid collection and transport of samples from suspect cases of AHS to a laboratory for AHS diagnosis as described in the Terrestrial Manual;
  - c) a system for recording, managing and analysing diagnostic, epidemiologic and surveillance data.
2. The AHS surveillance programme should:
  - a) in a country/zone, free or seasonally free, include an early warning system for reporting suspicious cases. Persons who have regular contact with equids, as well as diagnosticians,

should report promptly any suspicion of AHS to the Veterinary Authority. An effective surveillance system will periodically identify suspicious cases that require follow-up and investigation to confirm or exclude that the cause of the condition is AHS. The rate at which such suspicious cases are likely to occur will differ between epidemiological situations and cannot therefore be predicted reliably. All suspected cases of AHS should be investigated immediately and samples should be taken and submitted to a laboratory. This requires that sampling kits and other equipment are available for those responsible for surveillance;

- b) conduct random or targeted serological and virological surveillance appropriate to the infection status of the country or zone in accordance with Chapter 1.4.

Article 12.1.13.

### **Surveillance strategies**

The target population for surveillance aimed at identification of disease and/or infection should cover susceptible equids within the country or zone. Active and passive surveillance for AHSV infection should be ongoing. Surveillance should be composed of random or targeted approaches using virological, serological and clinical methods appropriate for the infection status of the country or zone.

A Member should justify the surveillance strategy chosen as appropriate to detect the presence of AHSV infection in accordance with Chapter 1.4. and the prevailing epidemiological situation. It may, for example, be appropriate to target clinical surveillance at particular species likely to exhibit clinical signs (e.g. horses). Similarly, virological and serological testing may be targeted to species that rarely show clinical signs (e.g. donkeys).

In vaccinated populations serological and virological surveillance is necessary to detect the AHSV types circulating to ensure that all circulating types are included in the vaccination programme.

If a Member wishes to declare freedom from AHSV infection in a specific zone, the design of the surveillance strategy would need to be aimed at the population within the zone.

For random surveys, the design of the sampling strategy will need to incorporate epidemiologically appropriate design prevalence. The sample size selected for testing will need to be large enough to detect infection if it were to occur at a predetermined minimum rate. The sample size, expected prevalence and diagnostic sensitivity of the tests determine the level of confidence in the results of the survey. The Member must justify the choice of design prevalence and confidence level based on the objectives of surveillance and the epidemiological situation, in accordance with Chapter 1.4. Selection of the design prevalence, in particular, needs to be based on the prevailing or historical epidemiological situation.

Irrespective of the survey approach selected, the sensitivity and specificity of the diagnostic tests employed are key factors in the design, sample size determination and interpretation of the results obtained. Ideally, the sensitivity and specificity of the tests used should be validated for the vaccination/infection history and the different species in the target population.

Irrespective of the testing system employed, surveillance system design should anticipate the occurrence of false positive reactions. If the characteristics of the testing system are known, the rate at which these false positives are likely to occur can be calculated in advance. There needs to be an effective procedure for following up positives to ultimately determine with a high level of confidence,

whether they are indicative of infection or not. This should involve both supplementary tests and follow-up investigation to collect diagnostic material from the original sampling unit as well as those which may be epidemiologically linked to it.

The principles for surveillance for disease/infection are technically well defined. Surveillance programmes to prove the absence of AHSV infection/circulation, need to be carefully designed to avoid producing results that are either insufficiently reliable to be accepted by international trading partners, or excessively costly and logistically complicated. The design of any surveillance programme, therefore, requires inputs from professionals competent and experienced in this field.

### 1. Clinical surveillance

Clinical surveillance aims at the detection of clinical signs of AHS in equids particularly during a newly introduced infection. In horses, clinical signs may include pyrexia, oedema, hyperaemia of mucosal membranes and dyspnoea.

AHS suspects detected by clinical surveillance should always be confirmed by laboratory testing.

### 2. Serological surveillance

Serological surveillance of equid populations is an important tool to confirm absence of AHSV transmission in a country or zone. The species tested should reflect the local epidemiology of AHSV infection, and the equine species available. Management variables that may reduce the likelihood of infection, such as the use of insecticides and animal housing, should be taken into account when selecting equids to be included in the surveillance system.

Samples should be examined for antibodies against AHSV using tests prescribed in the Terrestrial Manual. Positive AHSV antibody tests results can have four possible causes:

- a) natural infection with AHSV;
- b) vaccination against AHSV;
- c) maternal antibodies;
- d) positive results due to the lack of specificity of the test.

It may be possible to use sera collected for other purposes for AHSV surveillance. However, the principles of survey design described in these recommendations and the requirements for a statistically valid survey for the presence of AHSV infection should not be compromised.

The results of random or targeted serological surveys are important in providing reliable evidence that no AHSV infection is present in a country or zone. It is, therefore, essential that the survey is thoroughly documented. It is critical to interpret the results in light of the movement history of the animals being sampled.

Serological surveillance in a free zone should target those areas that are at highest risk of AHSV transmission, based on the results of previous surveillance and other information. This will usually be towards the boundaries of the free zone. In view of the epidemiology of AHSV, either random or targeted sampling is suitable to select herds and/or animals for testing.

Serological surveillance in a free country or zone should be carried out over an appropriate distance from the border with an infected country or infected zone, based upon geography, climate, history of infection and other relevant factors. The surveillance should be carried out over a distance of at least 100 kilometres from the border with that country or zone, but a lesser distance could be acceptable if there are relevant ecological or geographical features likely to interrupt the transmission of AHSV. An AHSV free country or zone may be protected from an adjacent infected country or infected zone by a ~~buffer~~ protection zone.

Serological surveillance in infected zones will identify changes in the boundary of the zone, and can also be used to identify the AHSV types circulating. In view of the epidemiology of AHSV infection, either random or targeted sampling is suitable.

### 3. Virological surveillance

Isolation and genetic analysis of AHSV from a proportion of infected animals is beneficial in terms of providing information on serotype and genetic characteristics of the viruses concerned.

Virological surveillance using tests described in the *Terrestrial Manual* can be conducted:

- a) to identify virus circulation in at risk populations;
- b) to confirm clinically suspect cases;
- c) to follow up positive serological results;
- d) to better characterize the genotype of circulating virus in a country or zone.

### 4. Sentinel animals

Sentinel animals are a form of targeted surveillance with a prospective study design. They comprise groups of unexposed equids that are not vaccinated and are managed at fixed locations and observed and sampled regularly to detect new AHSV infections.

The primary purpose of a sentinel equid programme is to detect AHSV infections occurring at a particular place, for instance sentinel groups may be located on the boundaries of infected zones to detect changes in distribution of AHSV. In addition, sentinel equid programmes allow the timing and dynamics of infections to be observed.

A sentinel equid programme should use animals of known source and history of exposure, control management variables such as use of insecticides and animal housing (depending on the epidemiology of AHSV in the area under consideration), and be flexible in its design in terms of sampling frequency and choice of tests.

Care is necessary in choosing the sites for the sentinel groups. The aim is to maximise the chance of detecting AHSV activity at the geographical location for which the sentinel site acts as a sampling point. The effect of secondary factors that may influence events at each location, such as climate, may also be analysed. To avoid confounding factors sentinel groups should comprise animals selected to be of similar age and susceptibility to AHSV infection. The only feature distinguishing groups of sentinels should be their geographical location. Sera from

sentinel animal programmes should be stored methodically in a serum bank to allow retrospective studies to be conducted in the event of new serotypes being isolated.

The frequency of sampling should reflect the equid species used and the reason for choosing the sampling site. In endemic areas virus isolation will allow monitoring of the serotypes and genotypes of AHSV circulating during each time period. The borders between infected and non infected areas can be defined by serological detection of infection. Monthly sampling intervals are frequently used. Sentinels in declared free zones add to confidence that AHSV infections are not occurring unobserved. Here sampling prior to and after the possible period of transmission is sufficient.

Definitive information on AHSV circulating in a country or zone is provided by isolation and identification of the viruses. If virus isolation is required sentinels should be sampled at sufficiently frequent intervals to ensure that some samples are collected during the period of viraemia.

#### 5. Vector surveillance

AHSV is transmitted between equine hosts by species of Culicoides which vary across the world. It is therefore important to be able to identify potential vector species accurately although many such species are closely related and difficult to differentiate with certainty.

The main purpose of vector surveillance is to define high, medium and low-risk areas and local details of seasonality by determining the various species present in an area, their respective seasonal occurrence, and abundance. Vector surveillance has particular relevance to potential areas of spread. Long term surveillance can also be used to assess vector abatement measures.

The most effective way of gathering this information should take account of the biology and behavioural characteristics of the local vector species of Culicoides and may include the use of Onderstepoort-type light traps or similar, operated from dusk to dawn in locations adjacent to equids.

Vector surveillance should be based on scientific sampling techniques. The choice of the number and types of traps to be used in vector surveillance and the frequency of their use should take into account the size and ecological characteristics of the area to be surveyed.

The operation of vector surveillance sites at the same locations as sentinel animals is advisable.

The use of a vector surveillance system to detect the presence of circulating virus is not recommended as a routine procedure as the typically low vector infection rates mean that such detections can be rare. Other surveillance strategies are preferred to detect virus circulation.