Annex 31

CHAPTER 12.1.

INFECTION WITH
AFRICAN HORSE SICKNESS VIRUS

Article 12.1.1.

General provisions

For the purposes of the *Terrestrial Code*, African horse sickness (AHS) is defined as an *infection* of equids with African horse sickness virus (AHSV).

The following defines the occurrence of an *infection* with AHSV:

1) AHSV has been isolated and identified as such in a sample from an equid ~~or a product derived from that equid~~; or

2) ~~antigen or ribo~~nucleic acid specific to AHSV has been ~~identified~~ detected in a sample~~s~~ from an equid showing clinical signs or pathological lesions consistent with AHS, or epidemiologically linked to a confirmed or suspected ~~or confirmed~~ *case*; or

3) ~~serological evidence of active~~*~~infection~~*~~with AHSV by detection of~~ seroconversion due to recent exposure to ~~with production of antibodies against structural or nonstructural proteins of~~ AHSV, ~~that are~~ which is not ~~a~~ the consequence of *vaccination*, ~~have~~ has been ~~identified~~ detected in ~~a~~ paired samples from an equid ~~that either~~ show~~s~~ing clinical signs or pathological lesions consistent with AHS, or ~~is~~ epidemiologically linked to a confirmed or suspected ~~or confirmed~~ *case.*

For the purposes of the *Terrestrial Code*, the *infective period* for AHS is 40 days. ~~for domestic horses. Although critical information is lacking for some species, this chapter applies to all Equidae..~~

All countries or *zones* adjacent to 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 Articles 12.1.11. to 12.1.13.~~

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

Article 12.1.2.

~~AHS free~~ ~~c~~Country or zone free from AHS

~~1)~~ A country or *zone* may be considered free from AHS when the relevant provisions in point 2 of Article 1.4.6.have been complied with, and when within the proposed free country or *zone*: *~~infection~~*~~with AHSV is notifiable in the whole country, systematic~~*~~vaccination~~*~~is prohibited, importation of equids and their semen, oocytes or embryos are carried out in accordance with this chapter, and either:~~

1) for at least the past 24 months:

a) the *Veterinary Authority* has current knowledge of, and authority over, all domestic and *captive wild* equids in the country or *zone*;

b) the *Veterinary Authority* has current knowledge of the distribution, habitat and indication of disease occurrence through passive *surveillance* of *wild* and *feral* equids in the country or *zone*;

c) ~~either:~~

~~i)~~ there has been no *case* of *infection* with AHSV ~~and the country or~~ *~~zone~~* ~~is not adjacent to an infected country or~~*~~zone~~*~~; or~~

~~ii)~~ ~~a~~*~~surveillance~~*~~programme has demonstrated no evidence of~~*~~Culicoides~~*~~in accordance with Chapter 1.5.~~;

d) appropriate *surveillance* has been implemented in accordance with:

i) Article 1.4.6. where historical freedom can be demonstrated; or

ii) Articles 12.1.11. to 12.1.13. where historical freedom cannot be demonstrated; or

iii) Chapter 1.5. where a *surveillance* programme has demonstrated no evidence of *Culicoides*.

e) if adjacent to an infected country or *zone,* include an area in which *surveillance* is conducted in accordance with Articles 12.1.11. to 12.1.13.;

f) measures to prevent the introduction of the *infection* have been in place: in particular, the importations or movements of *commodities* into the country or zone have been carried out in accordance with this chapter and other relevant chapters of the *Terrestrial Code*;

2) no systematic *vaccination* against AHS has been carried out for at least the past 12 months.

~~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 two years and is not adjacent to an infected country or~~*~~zone~~*~~; or~~

~~c)~~ ~~a~~*~~surveillance~~*~~programme has demonstrated no evidence of AHSV in the country or~~*~~zone~~*~~for at least two years; 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~~*~~for at least two years in the country or~~*~~zone~~*~~.~~

~~2)~~ ~~An AHS free country or~~*~~zone~~*~~which is adjacent to an infected country or~~*~~zone~~*~~should include a~~*~~zone~~*~~in which~~*~~surveillance~~*~~is conducted in accordance with Articles 12.1.11. to 12.1.13., as relevant.~~

~~3)~~ ~~An AHS free country or~~*~~zone~~*~~will not lose its free status through the importation of seropositive or vaccinated equids and their semen, oocytes or embryos from infected countries or~~*~~zones~~*~~, provided these imports are carried out in accordance with this chapter.~~

~~4)~~ ~~To qualify for inclusion in the list of AHS free countries or~~*~~zones~~*~~, a Member Country should:~~

~~a)~~ ~~have a record of regular and prompt animal disease reporting;~~

~~b)~~ ~~send a declaration to the OIE stating:~~

~~i)~~ ~~the section under point 1) on which the application is based;~~

~~ii)~~ ~~no routine~~*~~vaccination~~*~~against AHS has been carried out during the past year in the country or~~*~~zone~~*~~;~~

~~iii)~~ ~~equids are imported in accordance with this chapter;~~

~~c)~~ ~~supply documented evidence that:~~

~~i)~~ *~~surveillance~~*~~in accordance with Articles 12.1.11. to 12.1.13. is applied, unless historically free in accordance with Article 1.4.6.;~~

~~ii)~~ ~~regulatory measures for the early detection, prevention and control of~~*~~infection~~*~~with AHSV have been implemented.~~

~~5)~~ ~~The Member Country will be included in the list only after the submitted evidence has been accepted by the OIE.~~

The country or *zone* will be included in the list of countries or *zones* free from AHS in accordance with Chapter 1.6.

Retention on the list requires annual reconfirmation of compliance with all points above and ~~relevant~~ provisions under point 4 of Article 1.4.6. ~~that the information in points 4 b) ii) and iii) and 4 c) above be annually re-submitted and~~ Documented evidence should be resubmitted annually for point 1 above. Any changes in the epidemiological situation or other significant events should be ~~reported~~ notified to WOAH in accordance with ~~the requirements in~~ Chapter 1.1.~~, and in particular, formally state that:~~

~~a)~~ ~~there has been no~~*~~outbreak~~*~~of AHS during the past year in the country or~~*~~zone~~*~~;~~

~~b)~~ ~~no evidence of~~*~~infection~~*~~with AHSV has been found during the past year in the country or~~*~~zone~~*~~.~~

Article 12.1.3.

~~AHS infected~~ ~~c~~Country or zone infected with AHSV

A country or *zone* shall be considered as infected with AHSV ~~For the purposes of this chapter, an AHS infected country or~~*~~zone~~*~~is one that does not fulfil~~ when the requirements for acceptance as a country or *zone* free from AHS are not fulfilled ~~to qualify as AHS free~~.

Article 12.1.4.

Establishment of a containment zone within a ~~an AHS free~~ country or zone previously free from AHS

In the event of ~~limited~~ *outbreaks* of AHS within a~~n AHS free~~ country or *zone* previously free from AHS, including within a *protection zone*, a ~~single~~ *containment zone*, which includes all epidemiologically linked *outbreaks*, ~~can~~ may be established, in accordance with Article 4.4.7., ~~for the purpose of~~ to minimise~~ing~~ the impact on the ~~entire~~ rest of the country or *zone*. ~~Such a~~*~~zone~~*~~should include all~~*~~cases~~*~~and can be established within a~~*~~protection zone~~*~~.~~

For this to be achieved and for the Member Country to take full advantage of this process, the *Veterinary Authority* should ~~provide~~ submit as soon as possible to WOAH, in addition to the requirements of Article 4.4.7., ~~in support of the application,~~ documented evidence that:

1) the *outbreaks* have been contained ~~are limited~~ based on the following factors:

a) ~~immediately on suspicion, a rapid response has been implemented, including~~*~~notification~~*~~reporting, standstill of movements of equids and effective controls of the movements of equine~~ *~~commodities~~* ~~has been made~~on suspicion, a standstill has been imposed on the suspected *establishments* and effective controls on the movement of animals and other *commodities* are in place in the country or *zone*;

b) the *infection* has been confirmed and notified in accordance with Chapter 1.1.;

c~~b~~) ~~standstill of movements of equids has been imposed, and effective controls on the movement of equids and their products specified in this chapter are in place~~on confirmation, the standstill and movement controls described in point 1 have been reinforced;

~~c)~~ ~~epidemiological investigation (trace-back, trace-forward) has been completed;~~

~~cd) the~~*~~infection~~*~~has been confirmed and notified in accordance with Chapter 1.1;~~

d~~e~~) epidemiological investigations ~~on~~ into the likely source of the *outbreak* have been carried out;

~~f)~~ ~~all~~*~~cases~~*~~have been shown to be epidemiologically linked;~~

e~~g~~) no new *cases* have been found in the *containment zone* within a minimum of two *infective periods* as defined in Article 12.1.1.;

~~2)~~ t~~he equids within the~~*~~containment zone~~*~~are clearly identifiable as belonging to the~~*~~containment zone~~*~~;~~

2) increased passive and targeted *surveillance* in accordance with Articles 12.1.11. to 12.1.13. in the rest of the country or *zone* has not detected any evidence of *infection*;

3) ~~animal health~~ measures are in place to effectively prevent the spread of AHSV *infection* to the rest of the country or *zone*, taking into consideration the establishment of a *protection zone* within the *containment zone*, the seasonal *vector* conditions and existing physical, geographical and ecological barriers;

4) ongoing *surveillance* in accordance with Articles 12.1.11. to 12.1.13. is in place in the *containment zone*.

~~The free status of the areas outside the~~*~~containment zone~~*~~is suspended while the~~*~~containment zone~~*~~is being established in accordance with points 1) to 5) above.~~ The free status of the areas of outside the *containment zone* is suspended while the *containment zone* is being established. The free status of these areas ~~outside the~~*~~containment zone~~* may be reinstated irrespective of Article 12.1.5. once the *containment zone* has been approved ~~is recognised~~ by ~~the~~ WOAH as complying with points 1 to 4 above.

In the event of the recurrence of ~~AHSV~~ *infection* with AHSV in the *containment zone*, established in accordance with point 4(a) of Article 4.4.7., the approval of the *containment zone* is withdrawn and the ~~AHS~~ free status of the whole country or *zone* is suspended until the relevant requirements of Article 12.1.5. are fulfilled.

In the event of occurrence of *infection* with AHSV in the outer zone of a *containment zone* established in accordance with point 4(b) of Article 4.4.7., the approval of the *containment zone* is withdrawn and the free status of the whole country or *zone* is suspended until the relevant requirements of Article 12.1.5. are fulfilled.

The recovery of the ~~AHS~~ free status of the *containment zone* should follow Article 12.1.5.

Article 12.1.5.

Recovery of free status

~~To regain free status when an AHS~~*~~outbreak~~*~~occurs in a country or~~*~~zone~~*~~previously free, Article 12.1.2. applies, irrespective of whether emergency~~*~~vaccination~~*~~has been applied or not.~~

Should an *outbreak* of AHS occur in a previously free country or zone, its status may be recovered in accordance with Article 12.1.2., irrespective of whether emergency *vaccination* has been applied or not.

The AHS free status of the country or zone will be reinstated only after the submitted evidence has been accepted by ~~the~~ WOAH.

Article 12.1.6.

Recommendations for importation of equids from AHS free countries or zones

~~For equids~~

*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 AHS free country or *zone* since birth or for at least 40 days prior to shipment;

4) either:

a) did not transit through an infected *zone* during transportation to the *place of shipment*; or

b) were protected from *Culicoides* attacks at all times when transiting through an infected *zone*.

Article 12.1.7.

Recommendations for importation of equids from AHS infected countries or zones

~~For equids~~

*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 in isolation in a *vector*-protected *establishment*:

a) for a period of at least 28 days and a serological test to detect antibodies against ~~the~~ AHSV ~~group~~, was carried out with a negative result on a blood sample collected at least 28 days after introduction into the *vector*-protected *establishment*; or

b) for a period of at least 40 days and serological tests 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 *vector*-protected *establishment*; or

c) for a period of at least 14 days and a~~n~~ ~~agent identification~~ test for the ~~identification~~ detection of the agent was carried out with a negative result on a blood sample collected not less than 14 days after introduction into the *vector*-protected *establishment*; or

d) for a period of at least 40 days and were vaccinated, at least 40 days before shipment, against all serotypes whose presence in the source population has been demonstrated through a *surveillance* programme in accordance with Articles 12.1.12. and 12.1.13., and were identified in the accompanying certification as having been vaccinated;

4) were protected from *Culicoides* attacks at all times during transportation (including transportation to and at the *place of shipment*).

Article 12.1.8.

Recommendations for the importation of equine 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 ~~immunised~~ vaccinated against AHS with a live attenuated vaccine within 40 days prior to the day of collection;

3) were either:

a) kept in an AHS free country or [*zone*](https://www.oie.int/index.php?id=169&L=0&htmfile=glossaire.htm#terme_zone_region)for at least 40 days before commencement of, and during collection of the semen; or

b) kept in an AHS free *vector*-protected *artificial insemination centre* throughout the collection period, and subjected to either:

i) a serological test to detect antibodies against ~~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 for the ~~identification~~ detection of the agent carried out with negative results on blood samples collected at commencement and conclusion of, and at least every seven days, during semen collection for this consignment.

Article 12.1.9.

Recommendations for the importation of *in vivo* derived equine oocytes or embryos

*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 oocytes or embryos and for the following 40 days;

b) had not been ~~immunised~~ vaccinated against AHS with a live attenuated vaccine within 40 days prior to the day of collection;

c) were either:

i) kept in an AHS free country or *zone* for at least 40 days before commencement of, and during collection of the oocytes or embryos, or

ii) kept in an AHS free *vector*-protected *collection centre* throughout the collection period, and subjected to either:

‒ a serological test to detect antibodies against ~~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 oocytes or embryos; or

‒ ~~agent identification~~ tests for the ~~identification~~ detection of the agent carried out with negative results on blood samples collected at commencement and conclusion of, and at least every seven days during oocytes or embryos collection for this consignment;

2) the embryos were collected, processed and stored in accordance with Chapters 4.8. and 4.10., as relevant;

3) the semen used to fertilise the oocytes complies at least with the requirements in Article 12.1.8.

Article 12.1.10.

Protecting animals from *Culicoides* attacks

1. Vector-protected establishment or facility

The *establishment* or facility should be approved by the *Veterinary Authority* and the means of protection should at least comprise the following:

a) appropriate physical barriers at entry and exit points, for example double-door entry-exit system;

b) openings of the building are *vector* screened with mesh of appropriate gauge impregnated regularly with an approved insecticide in accordance with the instructions of the manufacturer;

c) *vector* *surveillance* and control within and around the building;

d) measures to limit or eliminate breeding sites for *vectors* in vicinity of the *establishment* or facility;

e) Standard Operating Procedure, including description of back-up and alarm systems, for operation of the *establishment* or facility and transport of equids to the place of *loading*.

2. During transportation

When equids are transport~~ing~~ed ~~equids~~ through AHS infected countries or *zones*, *Veterinary Authorities* should require that they are ~~strategies to~~ protected ~~animals~~ from *Culicoides* attacks ~~during transport~~, taking into account the local ecology of the *vector*.

a) Transport by ~~road~~ land

Potential *risk management* strategies include a combination of:

i) treating animals with chemical repellents prior to and during transportation, in sanitized *vehicles* treated with appropriate residual contact insecticide;

ii) *loading*, transporting and *unloading* animals at times of low *vector* activity (i.e. bright sunshine and low temperature);

iii) ensuring *vehicles* do not stop en route during dawn or dusk, or overnight, unless the *animals* are held behind insect proof netting;

iv) darkening the interior of the *vehicle*, for example by covering the roof or sides of *vehicles* with shade cloth;

v) surveillance for *vectors* at common stopping and offloading points to gain information on seasonal variations;

vi) using historical, ongoing or modelling information on AHS to identify low risk ports and transport routes.

b) Transport by air

Prior to *loading* the equids, the crates, *containers* or jet stalls are sprayed with an insecticide approved in the country of dispatch.

Crates, *containers* or jet stalls in which equids are being transported and the cargo hold of the aircraft should be sprayed with an approved insecticide when the doors have been closed and prior to take off. All possible insect harbourage should be treated. The spray containers should be retained for inspection on arrival.

In addition, during any stopover in countries or *zones* not free from AHS, prior to the opening of any aircraft door and until all doors are closed, netting of appropriate gauge impregnated with an approved insecticide should be placed over all crates, *containers* or jet stalls.

Article 12.1.11.

Introduction to surveillance

Articles 12.1.11. to 12.1.13. define the principles and provide guidance on *surveillance* for AHS, complementary to Chapter 1.4. and, for *vectors*, complementary to Chapter 1.5.

AHS is a *vector*-borne *infection* transmitted by ~~a limited number of~~ some 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~~A Member Country demonstrating freedom from *infection* with AHSV 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 in accordance with general conditions and methods described in this chapter. This requires the support of a *laboratory* able to undertake identification of *infection* with AHSV through the ~~virus detection~~ tests for the detection of the agent and antibody detection tests.

Susceptible *captive wild*, *feral* and *wild* equine populations should be included in the *surveillance* programme.

The purpose of *surveillance* is to determine if a country or *zone* is free from AHS. *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.

Article 12.1.12.

General conditions and methods for surveillance

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 suspected cases of AHS to a *laboratory* for diagnosis;

c) a system for recording, managing and analysing diagnostic, epidemiological and *surveillance* data.

2) In a free country or *zone*, the *surveillance* programme for AHS should include an *early warning system* for reporting suspected 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 suspected cases that require follow-up and investigation to confirm or exclude that the cause of the condition is AHS. The rate at which such suspected 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 be available to those responsible for *surveillance*.

3) In a free country or *zone* ~~bordering~~ adjacent to an infected country or *zone*, *surveillance* ~~based upon~~ taking into account geography, climate, history of infection and other relevant factors should be carried out over an appropriate distance of at least 100 kilometres from the border with the infected country or *zone*; lesser distance could be acceptable if there are relevant ecological or geographical features likely to interrupt the transmission of AHSV.

4) In an AHS infected country or *zone*, random or targeted serological and virological *surveillance*, appropriate to the epidemiological situation, should be conducted in accordance with Chapter 1.4.

Article 12.1.13.

Surveillance strategies

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

A Member Country should justify the *surveillance* strategy chosen as appropriate to detect the presence of *infection* with AHSV 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.

Serological or virological *surveillance* is also needed to detect subclinical *infections* in free countries or *zones* adjacent to countries or *zones* in which live attenuated AHS vaccines are used.

For random surveys, the design of the sampling strategy should incorporate epidemiologically appropriate design prevalence. The sample size selected for testing should 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 Country should 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, should 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* or *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 should 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 or *infection* are technically well defined. *Surveillance* programmes to prove the absence of AHSV *infection* or transmission, should be carefully designed to avoid producing results that are insufficiently reliable to be accepted by WOAH for official recognition of status. 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 mucous membranes and dyspnoea.

Suspected cases detected by clinical *surveillance* should always be confirmed by *laboratory* testing.

2. Serological surveillance

Serological *surveillance* of equine 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 *infection* with AHSV, and the equine species available. Surveillance plans should include consideration of species that display clinical signs less commonly, such as donkeys or zebra. 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. Positive AHSV antibody tests results can have four possible causes:

a) natural *infection* with AHSV;

b) *vaccination* against AHS;

c) maternal antibodies;

d) lack of specificity of the test.

Sera collected for other purposes may be used 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 *infection* with AHSV should not be compromised.

The results of random or targeted serological surveys are important in providing reliable evidence that no *infection* with AHSV 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* 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~~*~~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 AHS free country or~~*~~zone~~*~~may be protected from an adjacent infected country or~~*~~zone~~*~~by a~~*~~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 *infection* with AHSV, 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* can be conducted:

a) to identify virus transmission in at risk populations;

b) to confirm clinically suspected cases;

c) to follow up positive serological results;

d) to better characterise 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 have not been vaccinated and are managed at fixed locations and observed and tested regularly to detect new *infections* with AHSV.

The primary purpose of a sentinel equid programme is to detect *infections* with AHSV 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 *infection* with AHSV. 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 equine 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 *infections* with AHSV 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.

*Vector* *surveillance* is aimed at demonstrating the absence of *vectors* or defining 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 or to confirm continued absence of *vectors*.

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 viruses is not recommended as a routine procedure as the typically low *vector* *infection* rates mean that such detections can be rare. Animal-based *surveillance* strategies are preferred to detect virus transmission.

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