

## CHAPTER 10.4.

**INFECTION WITH HIGH PATHOGENICITY  
AVIAN INFLUENZA VIRUSES**

## Article 10.4.1.

## General provisions

- 1) The objective of this chapter is to mitigate animal and public health risks posed by avian influenza viruses, and prevent their international spread. The chapter focuses on high pathogenicity avian influenza viruses, which cause the listed disease of concern. However, since they have the ability to mutate into high pathogenicity viruses, low pathogenicity avian influenza viruses of H5 and H7 subtypes should be included in any surveillance and control programmes for high pathogenicity viruses. This chapter deals not only with the occurrence of clinical signs caused by avian influenza, but also with the presence of infection with avian influenza viruses in the absence of clinical signs.

For the purposes of the *Terrestrial Code*, avian influenza is defined as an *infection of poultry* caused by any influenza A virus of the H5 or H7 subtypes or by any influenza A virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. These viruses are divided into high pathogenicity avian influenza viruses and low pathogenicity avian influenza viruses:

- a) ~~high pathogenicity avian influenza viruses have an IVPI in six-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in four to eight week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other high pathogenicity avian influenza isolates, the isolate being tested should be considered as high pathogenicity avian influenza virus;~~
- b) ~~low pathogenicity avian influenza viruses are all influenza A viruses of H5 and H7 subtypes that are not high pathogenicity avian influenza viruses.~~
- 2) For the purposes of the *Terrestrial Code*:
- a) High pathogenicity avian influenza means an infection of poultry by any influenza A virus with an intravenous pathogenicity index (IVPI):
- = in six-week-old chickens greater than 1.2 or, as an alternative, causes at least 75% mortality in four to eight-week-old chickens infected intravenously. Viruses of H5 and H7 subtypes that do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other high pathogenicity avian influenza isolates, the isolate being tested should be considered as a high pathogenicity avian influenza virus.
- b) The following defines the occurrence of infection with a high pathogenicity avian influenza virus: the virus has been isolated and identified as such or specific viral ribonucleic acid has been detected in one or more samples from poultry or a product derived from poultry.
- 3) ~~*Poultry* is defined as 'all domesticated birds, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose'.~~

## Annex 19 (contd)

Birds that are kept in captivity for any reason other than those reasons referred to in the preceding paragraph, including those that are kept for shows, races, exhibitions, competitions or for breeding or selling these categories of birds as well as pet birds, are not considered to be *poultry*.

- c) *Poultry* means all domesticated birds used for the production of *meat* or eggs for consumption, for the production of other commercial products, or for breeding these categories of birds, as well as fighting cocks used for any purpose. All birds used for restocking supplies of game are considered *poultry*. If birds are kept in a single household and their products are only used in the same household, these birds are not considered *poultry*.

Comments: African Union seeks clarification on why household poultry are not considered as poultry.
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Birds that are kept in captivity for any reason other than those referred to in the preceding paragraph, including those that are kept for shows, races, exhibitions, competitions or for breeding or selling these categories of birds as well as pet birds, are not considered *poultry*.

- d) the *incubation period* at the *flock* level for high pathogenicity avian influenza shall be 14 days.
- 3) In accordance with Chapter 1.1., a sudden and unexpected change in the distribution, host range, or increase in incidence or virulence of, or morbidity or mortality caused by avian influenza viruses is notifiable to the OIE, as well as zoonotic avian influenza viruses. Occurrences of influenza A viruses of high pathogenicity in birds other than *poultry*, including *wild* birds, are notifiable. Six-monthly reports on the presence of avian influenza viruses in a country or *zone* should include low pathogenicity viruses of H5 and H7 subtypes.

A notification of infection with influenza A viruses of high pathogenicity in birds other than *poultry*, including *wild* birds, or of low pathogenicity avian influenza viruses in *poultry* does not affect the status of the country or *zone*. A Member Country should not impose bans on the trade in *poultry* and *poultry commodities* in response to such notification, or to other information on the presence of any influenza A virus in birds other than *poultry*, including *wild* birds.

For the purposes of the *Terrestrial Code*, the *incubation period* for avian influenza shall be 21 days.

- 5) ~~This chapter deals not only with the occurrence of clinical signs caused by avian influenza, but also with the presence of *infection* with avian influenza viruses in the absence of clinical signs.~~
- 6) ~~Antibodies against H5 or H7 subtype, which have been detected in *poultry* and are not a consequence of *vaccination*, should be immediately investigated. In the case of isolated serological positive results, *infection* with avian influenza viruses may be ruled out on the basis of a thorough epidemiological and *laboratory* investigation that does not demonstrate further evidence of such an *infection*.~~
- 7) ~~For the purposes of the *Terrestrial Code*, 'avian influenza free establishment' means an establishment in which the *poultry* have shown no evidence of infection with avian influenza viruses, based on surveillance in accordance with Articles 10.4.27. to 10.4.33.~~
- 8) ~~*Infection* with influenza A viruses of high pathogenicity in birds other than *poultry*, including *wild* birds, should be notified according to Article 1.1.3. However, a Member Country should not impose bans on the trade in *poultry* and *poultry commodities* in response to such a *notification*, or other information on the presence of any influenza A virus in birds other than *poultry*, including *wild* birds.~~
- 4) The use of *vaccination* against high pathogenicity avian influenza in *poultry* may be recommended under specified conditions, while not affecting the status of a free country or *zone* if the vaccine complies with the standards in the *Terrestrial Manual*. *Vaccination* is an effective complementary control tool that can be used when a *stamping-out policy* alone is not sufficient. The decision whether to vaccinate or not is to be made by the *Veterinary Authorities* based on the avian influenza situation as well as the ability of the *Veterinary Services*

to execute the proper vaccination strategy, as described in Chapter 4.17. Any vaccine used should comply with the standards described in the *Terrestrial Manual*.

Annex 19 (contd)

- 59) Standards for diagnostic tests and vaccines, including pathogenicity testing, are described in the *Terrestrial Manual*. ~~Any vaccine used should comply with the standards described in the *Terrestrial Manual*.~~

Article 10.4.1bis.

Safe commodities

When authorising import or transit of the following commodities, Veterinary Authorities should not require any avian influenza related conditions, regardless of the avian influenza status of the exporting country or zone:

- 1) heat-treated poultry meat in a hermetically sealed container with a F-value of 3.00 or above;
- 2) extruded dry pet food and poultry-based coated ingredients after extrusion;
- 3) rendered meat and bone meal, blood meal, feather meal, and poultry oil;
- 4) feathers and down from poultry and other birds processed by washing and steam-drying.

Other commodities of poultry and other birds can be traded safely if in accordance with the relevant articles of this chapter.

~~Article 10.4.2.~~

~~Determination of the avian influenza status of a country, zone or compartment~~

~~The avian influenza status of a country, a zone or a compartment can be determined on the basis of the following criteria:~~

- 1) ~~avian influenza is notifiable in the whole country, an ongoing avian influenza awareness programme is in place, and all notified suspect occurrences of avian influenza are subjected to field and, where applicable, laboratory investigations;~~
- 2) ~~appropriate surveillance is in place to demonstrate the presence of infection in the absence of clinical signs in poultry, and the risk posed by birds other than poultry; this may be achieved through an avian influenza surveillance programme in accordance with Articles 10.4.27. to 10.4.33.;~~
- 3) ~~consideration of all epidemiological factors for avian influenza occurrence and their historical perspective.~~

~~Article 10.4.3.~~

~~Country, zone or compartment free from avian influenza~~

~~A country, zone or compartment may be considered free from avian influenza when it has been shown that infection with avian influenza viruses in poultry has not been present in the country, zone or compartment for the past 12 months, based on surveillance in accordance with Articles 10.4.27. to 10.4.33.~~

~~If infection has occurred in poultry in a previously free country, zone or compartment, avian influenza free status can be regained:~~

- 1) ~~In the case of infections with high pathogenicity avian influenza viruses, three months after a stamping-out policy (including disinfection of all affected establishments) is applied, providing that surveillance in accordance with Articles 10.4.27. to 10.4.33. has been carried out during that three-month period.~~
- 2) ~~In the case of infections with low pathogenicity avian influenza viruses, poultry may be kept for slaughter for human consumption subject to conditions specified in Article 10.4.19. or a stamping-out policy may be applied; in either case, three months after the disinfection of all affected establishments, providing that surveillance in accordance with Articles 10.4.27. to 10.4.33. has been carried out during that three-month period.~~



## Annex 19 (contd)

## Article 10.4.34.

~~Country, or zone or compartment free from infection with high pathogenicity avian influenza viruses in poultry~~

A country, ~~or zone or compartment~~ may be considered free from ~~infection with~~ high pathogenicity avian influenza viruses ~~in poultry~~ when:

- = ~~infection with high pathogenicity avian influenza viruses in poultry is a notifiable disease in the entire country;~~
- = ~~an ongoing avian influenza surveillance is implemented to monitor the general situation of H5 and H7 low pathogenicity avian influenza viruses in poultry and an awareness programme is in place related to biosecurity and management of H5 and H7 low pathogenicity avian influenza viruses;~~
- = ~~4) based on surveillance in accordance with Chapter 1.4. and Articles 10.4.27. to 10.4.33., it has been shown demonstrated that infection with high pathogenicity avian influenza viruses in poultry as defined in Article 10.4.1. has not been present occurred in the country, or zone or compartment for the past 12 months; Although its status with respect to low pathogenicity avian influenza viruses may be unknown; or~~
- = ~~bird commodities are imported in accordance with Articles 10.4.5. to 10.4.23.~~

The ~~surveillance should~~ ~~may need to~~ be adapted to parts of the country or existing ~~zones or compartment~~ depending on historical or geographical factors, industry structure, population data, ~~or proximity to recent outbreaks or the use of vaccination.~~

If ~~infection has occurred in poultry in a previously free country, zone or compartment, the free status can be regained three months after a stamping-out policy (including disinfection of all affected establishments) is applied, providing that surveillance in accordance with Articles 10.4.27. to 10.4.33. has been carried out during that three-month period.~~

## Article 10.4.3bis.

Compartment free from high pathogenicity avian influenza

The establishment of a compartment free from high pathogenicity avian influenza should follow the relevant requirements of this chapter and the principles in Chapters 4.3. and 4.4.

Comment: The section should not only emphasize presence of HPAI in compartments. The presence of LPAI in a compartment is also not desirable. A compartment should be robust to exclude all influenza viruses. The OIE should consider other avenues to deal with the potential trade restrictions and not necessarily compromise the science.

## Article 10.4.3ter.

Establishment of a containment zone within a country or zone free from high pathogenicity avian influenza

In the event of outbreaks of high pathogenicity avian influenza within a previously free country or zone, a containment zone, which includes all epidemiologically linked outbreaks, may be established for the purposes of minimising the impact on the rest of the country or zone.

In addition to the requirements for the establishment of a containment zone outlined in Article 4.3.7., the surveillance programme should take into account the density of poultry production, types of poultry, local management practices (including inter-premise movement pattern of poultry, people and equipment), relevant biosecurity and presence and potential role of birds other than poultry, including wild birds and the proximity of poultry establishments to perennial and seasonal water bodies.

The free status of the areas outside the *containment zone* is suspended while the *containment zone* is being established. It may be reinstated irrespective of the provisions of Article 10.4.3quater., once the *containment zone* is clearly established. It should be demonstrated that *commodities for international trade* either have originated outside the *containment zone* or comply with the relevant articles of this chapter.

Article 10.4.3quater.**Recovery of free status**

If infection has occurred in poultry in a previously free country or zone, the free status can be regained after a minimum period of 28 days after a stamping-out policy has been completed, provided that surveillance in accordance with Articles 10.4.27. to 10.4.33., in particular point 3) of Article 10.4.30., has been carried out during that period and has demonstrated the absence of infection.

If a stamping-out policy is not implemented, Article 10.4.3. applies.

## Article 10.4.5.

**Recommendations for importation from a country, zone or compartment free from high pathogenicity avian influenza****For live poultry (other than day-old poultry)**

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

- 1) the *poultry* showed no clinical signs of avian influenza on the day of shipment;
- 2) a) the poultry were kept in originated from an avian influenza free a country, zone or compartment free from high pathogenicity avian influenza since they were hatched or for at least the past 21 days;  
b) the poultry originated from a flock free from infection with any H5 or H7 influenza A viruses;
- 3) the *poultry* are transported in new or appropriately sanitized *containers*.

If the *poultry* have been vaccinated against avian influenza, the nature of the vaccine used and the date of *vaccination* should be ~~attached to~~ mentioned in the *international veterinary certificate*.

## Article 10.4.6.

**Recommendations for the importation of live birds other than poultry**

Regardless of the ~~avian influenza~~ status of the country of origin, Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:

- 1) on the day of shipment, the birds showed no clinical signs of *infection* with a virus which would be considered avian influenza in *poultry*;
- 2) the birds were kept in isolation approved by the *Veterinary Services* since they were hatched or for at least 24 ~~28~~ days prior to shipment and showed no clinical signs of *infection* with a virus which would be considered avian influenza in *poultry* during the isolation period;
- 3) a statistically valid sample of the birds, selected in accordance with the provisions of Article 10.4.29., was subjected to a diagnostic test for influenza A viruses within 14 days prior to shipment, with negative results for H5 and H7 ~~to demonstrate freedom from infection with a virus which would be considered aviana influenza in poultry;~~
- 4) the birds are transported in new or appropriately sanitized *containers*.

If the birds have been vaccinated against avian influenza, the nature of the vaccine used and the date of *vaccination* should be ~~attached to~~ mentioned in the *international veterinary certificate*.

Annex 19 (contd)~~Article 10.4.7.~~~~Recommendations for importation from a country, zone or compartment free from avian influenza~~~~For day-old live poultry~~~~Veterinary Authorities should require the presentation of an international veterinary certificate attesting that:~~

- ~~1) the poultry were kept in an avian influenza free country, zone or compartment since they were hatched;~~
- ~~2) the poultry were derived from parent flocks which had been kept in an avian influenza free country, zone or compartment for at least 21 days prior to and at the time of the collection of the eggs;~~
- ~~3) the poultry are transported in new or appropriately sanitized containers.~~

~~If the poultry or the parent flocks have been vaccinated against avian influenza, the nature of the vaccine used and the date of vaccination should be attached to the certificate.~~

## Article 10.4.8.

~~Recommendations for importation from a country, zone or compartment free from infection with high pathogenicity avian influenza viruses in poultry~~~~For day-old live poultry~~~~Veterinary Authorities should require the presentation of an international veterinary certificate attesting that:~~

- ~~1) the poultry were kept in a country, zone or compartment free from infection with high pathogenicity avian influenza since they were hatched;~~
- ~~2) a) the poultry were derived from parent flocks free from infection with any H5 or H7 influenza A viruses which had been kept in an avian influenza free establishment for at least 21 days prior to and at the time of the collection of the eggs from which the day-old poultry hatched; or~~  
~~b) the day-old live poultry that hatched from eggs that have had their surfaces sanitized in accordance with point 4 d) of Article 6.5.5.;~~
- ~~2) the poultry are transported in new or appropriately sanitized containers.~~

~~If the poultry or the parent flocks have been vaccinated against avian influenza, the nature of the vaccine used and the date of vaccination should be attached to mentioned in the international veterinary certificate.~~

## Article 10.4.9.

~~Recommendations for the importation of day-old live birds other than poultry~~~~Regardless of the avian influenza status of the country of origin, Veterinary Authorities should require the presentation of an international veterinary certificate attesting that:~~

- ~~1) on the day of shipment, the birds showed no clinical signs of infection with a virus which would be considered avian influenza in poultry;~~
- ~~2) the birds were hatched and kept in isolation approved by the Veterinary Services;~~
- ~~3) the parent flock birds were subjected to a diagnostic test for influenza A viruses at the time of the collection of the eggs, with negative results for H5 and H7 to demonstrate freedom from infection with a virus which would be considered avian influenza in poultry;~~

- 4) the birds are transported in new or appropriately sanitized *containers*.

Annex 19 (contd)

If the birds or parent *flocks* have been vaccinated against avian influenza, the nature of the vaccine used and the date of *vaccination* should be ~~attached to~~ mentioned in the *international veterinary certificate*.

~~Article 10.4.10.~~

~~Recommendations for importation from a country, zone or compartment free from avian influenza~~

For hatching eggs of poultry

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

- 1) ~~the eggs came from an avian influenza free country, zone or compartment;~~
- 2) ~~the eggs were derived from parent flocks which had been kept in an avian influenza free country, zone or compartment for at least 21 days prior to and at the time of the collection of the eggs;~~
- 3) ~~the eggs are transported in new or appropriately sanitized packaging materials.~~

~~If the parent flocks have been vaccinated against avian influenza, the nature of the vaccine used and the date of vaccination should be attached to the certificate.~~

Article 10.4.11.

~~Recommendations for importation from a country, zone or compartment free from infection with high pathogenicity avian influenza viruses in poultry~~

For hatching eggs of poultry

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

- 1) the eggs came from a country, *zone* or *compartment* free from ~~infection with~~ high pathogenicity avian influenza viruses in *poultry*;
- 2) a) the eggs were derived from parent *flocks* free from infection with any H5 or H7 influenza A viruses which had been kept in an avian influenza free ~~establishment~~ for at least 21 days prior to and at the time of the collection of the eggs; or
  - b3) the eggs have had their surfaces sanitized (in accordance with ~~Chapter 6.5. point 4 d) of Article 6.5.5.~~);
- 34) the eggs are transported in new or appropriately sanitized packaging materials.

If the parent *flocks* have been vaccinated against avian influenza, the nature of the vaccine used and the date of *vaccination* should be ~~attached to~~ mentioned in the *international veterinary certificate*.

Article 10.4.12.

~~Recommendations for the importation of hatching eggs from birds other than poultry~~

Regardless of the ~~avian influenza~~ status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

- 1) a statistically valid sample of birds from the parent *flock* ~~birds were~~ was subjected to a diagnostic test for influenza A viruses ~~seven 14~~ days prior to and at the time of the collection of the eggs, with negative results for H5 and H7 to demonstrate freedom from ~~infection with a virus which would be considered avian influenza in poultry~~;

- 2) the eggs have had their surfaces sanitized (in accordance with point 4 d) of Article 6.5.5. Chapter 6.5.;

Annex 19 (contd)

- 3) the eggs are transported in new or appropriately sanitized packaging materials.

If the parent *flocks* have been vaccinated against avian influenza, the nature of the vaccine used and the date of vaccination should be ~~attached to~~ mentioned in the international veterinary certificate.

~~Article 10.4.13.~~

~~Recommendations for importation from a country, zone or compartment free from avian influenza~~

For eggs for human consumption

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

- ~~1) the eggs were produced and packed in an avian influenza free country, zone or compartment;~~
- ~~2) the eggs are transported in new or appropriately sanitized packaging materials.~~

Article 10.4.14.

~~Recommendations for importation from a country, zone or compartment free from infection with high pathogenicity avian influenza viruses in poultry~~

For eggs for human consumption

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

- 1) the eggs were produced and packed in a country, zone or *compartment* free from ~~infection with~~ high pathogenicity avian influenza ~~viruses in poultry~~;
- 2) the eggs have had their surfaces sanitized (in accordance with Chapter 6.5.);
- 3) the eggs are transported in new or appropriately sanitized packaging materials.

Article 10.4.15.

**Recommendations for importation of egg products of poultry**

Regardless of the ~~avian influenza~~ status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

- 1) the *commodity* is derived from eggs which meet the requirements of ~~Articles 10.4.13. or~~ 10.4.14. or
- 2) the *commodity* has been processed to ensure the ~~destruction~~ inactivation of high pathogenicity avian influenza virus in accordance with Article 10.4.25.;

AND

- 3) the necessary precautions were taken to avoid contact of the *commodity* with any source of high pathogenicity avian influenza virus.

~~Article 10.4.16.~~

~~Recommendations for importation from a country, zone or compartment free from avian influenza~~

For poultry semen

~~Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that the donor *poultry*:~~

Annex 19 (contd)

- ~~1) showed no clinical sign of avian influenza on the day of semen collection;~~
- ~~2) were kept in an avian influenza free country, *zone* or *compartment* for at least 21 days prior to and at the time of semen collection.~~

Article 10.4.17.

**Recommendations for the importation from a country, zone or compartment free from ~~infection with high pathogenicity avian influenza viruses in poultry~~**

For poultry semen

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

- 1) showed no clinical signs of ~~infection with high pathogenicity avian influenza viruses in poultry~~ on the day of semen collection;
- 2) were kept in a country, *zone* or *compartment* free from ~~infection with high pathogenicity avian influenza viruses in poultry~~ for at least 21 days prior to and at the time of semen collection.

Article 10.4.18.

**Recommendations for the importation of semen of birds other than poultry**

Regardless of the ~~avian influenza~~ status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the donor birds:

- 1) were kept in isolation approved by the *Veterinary Services* for at least ~~24~~ 28 days prior to semen collection;
- 2) showed no clinical signs of *infection* with a virus which would be considered avian influenza in *poultry* during the isolation period;
- 3) were tested within 14 days prior to semen collection and shown to be free from *infection* with a virus which would be considered avian influenza in *poultry*.

Article 10.4.19.

**Recommendations for importation from a country, zone or compartment ~~free from avian influenza or free from infection with high pathogenicity avian influenza viruses in poultry~~**

For fresh meat of poultry

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the entire consignment of *fresh meat* comes from *poultry*:

- 1) which ~~have been kept in~~ originated from a country, *zone* or *compartment* free from ~~infection with high pathogenicity avian influenza viruses in poultry since they were hatched or for at least the past 21 days;~~
- 2) which have been slaughtered in an approved *abattoir* in a country, *zone* or *compartment* free from ~~infection with high pathogenicity avian influenza viruses in poultry~~ and have been subjected to ante- and post-mortem inspections in accordance with Chapter 6.3. ~~and have been found free of any signs suggestive of avian influenza with favorable results.~~

Annex 19 (contd)

## Article 10.4.20.

**Recommendations for the importation of meat products of poultry**

Regardless of the ~~avian influenza~~ status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

- 1) the *commodity* is derived from *fresh meat* which meets the requirements of Article 10.4.19.; or
- 2) the *commodity* has been processed to ensure the ~~destruction~~ inactivation of high pathogenicity avian influenza virus in accordance with Article 10.4.26.;

AND

- 3) the necessary precautions were taken to avoid contact of the *commodity* with any source of high pathogenicity avian influenza virus.

Article 10.4.21.**Recommendations for the importation of poultry products not listed in Article 10.4.1bis and intended for use in animal feeding, or for agricultural or industrial use**

Regardless of the status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

- 1) these commodities were processed in a country, zone or compartment free from high pathogenicity avian influenza and from poultry which originated in a country, zone or compartment free from high pathogenicity avian influenza; or
- 2) these commodities have been processed to ensure the inactivation of high pathogenicity avian influenza virus using:
  - a) moist heat treatment for 30 minutes at 56 °C; or
  - b) heat treatment where the internal temperature throughout the product reaches at least 74 °C; or
  - c) any equivalent treatment that has been demonstrated to inactivate avian influenza virus;

AND

- 3) the necessary precautions were taken to avoid contact of the commodity with any source of high pathogenicity avian influenza virus.

~~Article 10.4.21.~~~~**Recommendations for the importation of products of poultry origin, other than feather meal and poultry meal, intended for use in animal feeding, or for agricultural or industrial use**~~

Regardless of the ~~avian influenza~~ status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

- 1) ~~these commodities were processed in an avian influenza free country, zone or compartment from poultry which were kept in an avian influenza free country, zone or compartment from the time they were hatched until the time of slaughter or for at least the 21 days preceding slaughter; or~~
- 2) ~~these commodities have been processed to ensure the destruction of avian influenza virus using:~~
  - a) ~~moist heat treatment for 30 minutes at 56°C; or~~

- ~~b) any equivalent treatment which has been demonstrated to inactivate avian influenza virus;~~

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AND

- ~~3) the necessary precautions were taken to avoid contact of the commodity with any source of avian influenza virus.~~

Article 10.4.22.

**Recommendations for the importation of feathers and down of poultry not listed in Article 10.4.1bis.**

~~Regardless of the avian influenza status of the country of origin,~~ *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

- 1) these *commodities* originated from *poultry* as described in Article 10.4.19. and were processed in an ~~avian influenza free~~ a country, zone or compartment free from high pathogenicity avian influenza; or
- 2) these *commodities* have been processed to ensure the inactivation of high pathogenicity avian influenza virus using one of the following:
  - a) ~~washed and steam dried at 100°C for 30 minutes;~~
  - ~~b) fumigation with formalin (10% formaldehyde) for 8 hours;~~
  - ~~b<sub>e</sub>) irradiation with a dose of 20 kGy;~~
  - ~~c<sub>d</sub>) any equivalent treatment which has been demonstrated to inactivate avian influenza virus;~~

AND

- ~~3) the necessary precautions were taken to avoid contact of the commodity with any source of high pathogenicity avian influenza virus.~~

Article 10.4.23.

**Recommendations for the importation of feathers and down of birds other than poultry**

Regardless of the ~~avian influenza~~ status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

- 1) these *commodities* have been processed to ensure the ~~destruction~~ inactivation of any virus which would be considered high pathogenicity avian influenza in *poultry* using one of the following:
  - a) ~~washed and steam dried at 100°C for 30 minutes;~~
  - ~~b) fumigation with formalin (10% formaldehyde) for 8 hours;~~
  - ~~b<sub>e</sub>) irradiation with a dose of 20 kGy;~~
  - ~~c<sub>d</sub>) any equivalent treatment which has been demonstrated to inactivate avian influenza virus;~~
- 2) the necessary precautions were taken to avoid contact of the *commodity* with any source of viruses which would be considered high pathogenicity avian influenza in *poultry*.

~~Article 10.4.24.~~

~~**Recommendations for the importation of feather meal and poultry meal**~~

Regardless of the avian influenza status of the country of origin, ~~Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:~~

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- ~~1) these commodities were processed in an avian influenza free country, zone or compartment from poultry which were kept in an avian influenza free country, zone or compartment from the time they were hatched until the time of slaughter or for at least the 21 days preceding slaughter, or~~
- 2) these commodities have been processed either:
  - a) with moist heat at a minimum temperature of 118°C for minimum of 40 minutes; or
  - b) with a continuous hydrolysing process under at least 3.79 bar of pressure with steam at a minimum temperature of 122°C for a minimum of 15 minutes; or
  - c) with an alternative rendering process that ensures that the internal temperature throughout the product reaches at least 74°C;

AND

- ~~3) the necessary precautions were taken to avoid contact of the commodity with any source of avian influenza viruses.~~

Article 10.4.25.

**Procedures for the inactivation of high pathogenicity avian influenza viruses in eggs and egg products**

The following times for industry standard temperatures are suitable for the inactivation of high pathogenicity avian influenza viruses present in eggs and egg products:

	Core temperature (°C)	Time
Whole egg	60	188 seconds
Whole egg blends	60	188 seconds
Whole egg blends	61.1	94 seconds
Liquid egg white	55.6	870 seconds
Liquid egg white	56.7	232 seconds
Plain or pure egg yolk	60	288 seconds
10% salted yolk	62.2	138 seconds
Dried egg white	67	20 hours
Dried egg white	54.4	50.4 hours
Dried egg white	51.7	73.2 hours

The listed temperatures are indicative of a range that achieves a 7-log kill of avian influenza virus. These are listed as examples in a variety of egg products, but when scientifically documented, variances from these times and temperatures and for additional egg products may also be suitable when they achieve equivalent inactivation of the virus.

Article 10.4.26.

**Procedures for the inactivation of high pathogenicity avian influenza viruses in meat**

The following times for industry standard temperatures are suitable for the inactivation of high pathogenicity avian influenza viruses.



	Core temperature (°C)	Time
Poultry meat	60.0	507 seconds
	65.0	42 seconds
	70.0	3.5 seconds
	73.9	0.51 second

The listed temperatures are indicative of a range that achieves a 7-log kill. Where scientifically documented, variances from these times and temperatures may also be suitable when they achieve the inactivation of the virus.

Article 10.4.26bis.

**Procedures for the inactivation of high pathogenicity avian influenza viruses in scientific specimens and skins and trophies**

For the inactivation of high pathogenicity avian influenza virus in scientific specimens and skins and trophies, one of the following procedures should be used:

- 1) boiling in water for an appropriate time so as to ensure that any matter other than bone, claws or beaks is removed; or
- 2) soaking, with agitation, in a 4% (w/v) solution of washing soda (sodium carbonate-Na<sub>2</sub>CO<sub>3</sub>) maintained at pH 11.5 or above for at least 48 hours; or
- 3) soaking, with agitation, in a formic acid solution (100 kg salt [NaCl] and 12 kg formic acid per 1,000 litres water) maintained below pH 3.0 for at least 48 hours; wetting and dressing agents may be added; or
- 4) in the case of raw hides, treating for at least 28 days with salt (NaCl) containing 2% washing soda (sodium carbonate-Na<sub>2</sub>CO<sub>3</sub>); or
- 5) treatment with 1% formalin for a minimum of six days;
- 6) any equivalent treatment which has been demonstrated to inactivate the virus.

Comments: African Union recommends that the sections (10.4.15, 10.4.20, 10.4.22 and 10.4.23) on inactivation procedures should take into account all influenza viruses and not only HPAI.

Article 10.4.27.

**Introduction to surveillance of high pathogenicity avian influenza**

Articles 10.4.27. to 10.4.33. define the principles and provide a guide on the *surveillance* for avian influenza complementary to Chapter 1.4., applicable to Member Countries seeking to determine their high pathogenicity avian influenza status. Surveillance is also necessary to support vaccination programmes, to monitor general situation of H5 and H7 low pathogenicity avian influenza viruses in poultry and for monitoring avian influenza in wild birds. This may be for the entire country, *zone* or *compartment*. Guidance for Member Countries seeking free status following an outbreak and for the maintenance of avian influenza status is also provided.

The presence of influenza A viruses in *wild birds* creates a particular problem. In essence, no Member Country can declare itself free from influenza A in *wild birds*. However, the definition of avian influenza in this chapter refers to the *infection in poultry only*, and Articles 10.4.27. to 10.4.33. were developed under this definition.

The impact and epidemiology of avian influenza differ widely in different regions of the world and therefore it is impossible to provide specific detailed recommendations for all situations. ~~Surveillance strategies employed for demonstrating freedom from avian influenza at an acceptable level of confidence should be adapted to the local situation.~~ Variables such as the frequency of contacts of *poultry* with *wild* birds, different biosecurity levels and production systems and the commingling of different susceptible species including domestic waterfowl require specific *surveillance* strategies to address each specific situation. It is incumbent upon the Member Country to provide scientific data that explains the epidemiology of avian influenza in the region concerned and also demonstrates how all the risk factors are managed. ~~There is therefore considerable latitude available to Member Countries to provide a well-reasoned argument to prove that absence of infection with avian influenza viruses is assured at an acceptable level of confidence.~~ Surveillance of H5 and H7 low pathogenicity avian influenza viruses in poultry is relevant as they might mutate into high pathogenicity viruses. There is currently no scientific evidence

#### Annex 19 (contd)

to predict if and when mutation might occur. Outbreaks of low pathogenicity viruses can be managed at establishment level, however spread to other poultry establishments increases the risk of virus mutation, in particular if it is not detected and managed. Therefore, a surveillance system should be in place to detect clusters of infected poultry establishments where H5 and H7 low pathogenicity viruses spread between poultry establishments.

~~Surveillance for avian influenza should be in the form of a continuing programme designed to establish that the country, zone or compartment, for which application is made, is free from infection with avian influenza viruses.~~

In cases where potential public health implications are suspected, reporting to the appropriate public health authorities is essential.

#### Article 10.4.28.

#### General conditions and methods for surveillance Surveillance for early warning of high pathogenicity avian influenza

- 1) Surveillance for avian influenza should be in the form of a continuing programme designed to detect the presence of infection with high pathogenicity avian influenza viruses in the country or zone in a timely manner. A surveillance system in accordance with Chapter 1.4. should be under the responsibility of the Veterinary Authority. In particular:
  - a) ~~a formal and ongoing system for detecting and investigating outbreaks of disease or infection with avian influenza viruses should be in place;~~
  - b) ~~a procedure should be in place for the rapid collection and transport of samples from suspect cases of avian influenza to a laboratory for avian influenza diagnosis;~~
  - c) ~~a system for recording, managing and analysing diagnostic and surveillance data should be in place.~~
- 2) The high pathogenicity avian influenza *surveillance* programme should:
  - a) include an early warning system in accordance with Article 1.4.5. throughout the production, marketing and processing chain for reporting suspicious suspected cases. Farmers and workers, who have day-to-day contact with *poultry*, as well as diagnosticians, should report promptly any suspicion of high pathogenicity avian influenza to the *Veterinary Authority*. ~~They should be supported directly or indirectly (e.g. through private veterinarians or veterinary para-professionals) by government information programmes and the Veterinary Authority.~~ All suspected cases of high pathogenicity avian influenza should be investigated immediately. As suspicion cannot always be resolved by epidemiological and clinical investigation alone, samples should be taken and submitted to a *laboratory* for appropriate tests. ~~This requires that sampling kits and other equipment are available for those responsible for surveillance. Personnel responsible for surveillance should be able to call for assistance from a team with expertise in avian influenza diagnosis and control. In cases where potential public health implications are suspected, notification to the appropriate public health authorities is essential;~~
  - b) implement, when as relevant, regular and frequent clinical inspection, and or serological and virological testing of high-risk groups of *animals*, such as those adjacent to an high pathogenicity avian influenza infected country or *zone*, places where birds and *poultry* of different origins are mixed, such as live bird markets, *poultry* in close proximity to waterfowl or other potential sources of influenza A viruses. This

activity is particularly applicable to domestic waterfowl where detection of high pathogenicity avian influenza via clinical suspicion can be of low sensitivity;

- c) ensure that antibodies against influenza A viruses, which have been detected in poultry and are not a consequence of vaccination, be immediately investigated. In the case of isolated serological positive results, infection with high pathogenicity avian influenza viruses may be ruled out on the basis of a thorough epidemiological and laboratory investigation that does not demonstrate further evidence of such an infection.

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 influenza A viruses. The rate at which such suspicious cases are likely to occur will differ between epidemiological situations and cannot therefore be predicted reliably. Documentation for freedom from *infection* with avian influenza viruses should, in consequence, provide details of the occurrence of suspicious cases and how they were investigated and dealt with. This should include the results of *laboratory* testing and the control measures to which the *animals* concerned were subjected during the investigation (quarantine, movement stand-still orders, etc.).

#### Article 10.4.29.

### ~~Surveillance strategies~~

#### 4. Introduction

The target population for *surveillance* aimed at identification of *disease* and *infection* should cover all the susceptible *poultry* species within the country, *zone* or *compartment*. Active and passive *surveillance* for avian influenza should be ongoing with the frequency of active *surveillance* being appropriate to the epidemiological situation in the country. *Surveillance* should be composed of random and targeted approaches using molecular, virological, serological and clinical methods.

The strategy employed may be based on randomised sampling requiring *surveillance* consistent with demonstrating the absence of *infection* with avian influenza viruses at an acceptable level of confidence. Random *surveillance* is conducted using serological tests. Positive serological results should be followed up with molecular or virological methods.

Targeted *surveillance* (e.g. based on the increased likelihood of *infection* in particular localities or species) may be an appropriate strategy. Virological and serological methods should be used concurrently to define the avian influenza status of high risk populations.

A Member Country should justify the *surveillance* strategy chosen as adequate to detect the presence of *infection* with avian influenza viruses in accordance with Chapter 1.4. and the prevailing epidemiological situation, including cases of high pathogenicity influenza A detected in any birds. It may, for example, be appropriate to target clinical *surveillance* at particular species likely to exhibit clear clinical signs (e.g. chickens). Similarly, virological and serological testing could be targeted to species that may not show clinical signs (e.g. ducks).

If a Member Country wishes to declare freedom from *infection* with avian influenza viruses in a specific *zone* or *compartment*, the design of the survey and the basis for the sampling process would need to be aimed at the population within the *zone* or *compartment*.

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 and expected *disease* prevalence 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 clearly 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* and *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 *flocks* which may be epidemiologically linked to it.

The principles involved in *surveillance* for *disease* and *infection* are technically well defined. The design of *surveillance* programmes to prove the absence of *infection* with, or circulation of, avian influenza viruses should be carefully followed to avoid producing results that are either insufficiently reliable, or excessively costly and logistically complicated. The design of any *surveillance* programme, therefore, requires inputs from professionals competent and experienced in this field.

## 2- Clinical surveillance

Clinical *surveillance* aims at the detection of clinical signs of avian influenza at the *flock* level. Whereas significant emphasis is placed on the diagnostic value of mass serological screening, *surveillance* based on clinical inspection should not be underrated. Monitoring of production parameters, such as increased mortality, reduced feed and water consumption, presence of clinical signs of a respiratory *disease* or a drop in egg production, is important for the early detection of *infection* with avian influenza viruses. In some cases, the only indication of *infection* with low pathogenicity avian influenza virus may be a drop in feed consumption or egg production.

Clinical *surveillance* and *laboratory* testing should always be applied in series to clarify the status of avian influenza suspects detected by either of these complementary diagnostic approaches. *Laboratory* testing may confirm clinical suspicion, while clinical *surveillance* may contribute to confirmation of positive serology. Any sampling unit within which suspicious *animals* are detected should have restrictions imposed upon it until avian influenza *infection* is ruled out.

Identification of suspect *flocks* is vital to the identification of sources of avian influenza viruses and to enable the molecular, antigenic and other biological characteristics of the virus to be determined. It is essential that avian influenza virus isolates are sent regularly to the regional Reference Laboratory for genetic and antigenic characterisation.

## 3- Virological surveillance

Virological *surveillance* should be conducted:

- a) to monitor at risk populations;
- b) to confirm clinically suspect cases;
- e) to follow up positive serological results;
- d) to test 'normal' daily mortality, to ensure early detection of *infection* in the face of *vaccination* or in *establishments* epidemiologically linked to an *outbreak*.

## 4- Serological surveillance

Serological *surveillance* aims at the detection of antibodies against avian influenza virus. Positive avian influenza viruses antibody test results can have four possible causes:

- a) natural *infection* with avian influenza viruses;
- b) *vaccination* against avian influenza;
- e) maternal antibodies derived from a vaccinated or infected parent *flock* are usually found in the yolk and can persist in progeny for up to four weeks;
- d) lack of specificity of the test.

It may be possible to use serum collected for other survey purposes for avian influenza *surveillance*. However, the principles of survey design described in these recommendations and the requirement for a statistically valid survey for the presence of avian influenza viruses should not be compromised.

The discovery of clusters of seropositive *flocks* may reflect any of a series of events, including but not limited to the demographics of the population sampled, vaccinal exposure or *infection*. As clustering may signal *infection*, the investigation of all instances should be incorporated in the survey design. Clustering of positive *flocks* is always epidemiologically significant and therefore should be investigated.

Annex 19 (contd)

If *vaccination* cannot be excluded as the cause of positive serological reactions, diagnostic methods to differentiate antibodies due to *infection* or *vaccination* should be employed.

The results of random or targeted serological surveys are important in providing reliable evidence that no *infection* with avian influenza viruses is present in a country, *zone* or *compartment*. It is therefore essential that the survey be thoroughly documented.

#### 5- Virological and serological surveillance in vaccinated populations

The *surveillance* strategy is dependent on the type of vaccine used. The protection against influenza A virus is haemagglutinin subtype specific. Therefore, two broad *vaccination* strategies exist: 1) inactivated whole viruses, and 2) haemagglutinin expression based vaccines.

In the case of vaccinated populations, the *surveillance* strategy should be based on virological or serological methods and clinical *surveillance*. It may be appropriate to use sentinel birds for this purpose. These birds should be unvaccinated, virus antibody free birds and clearly and permanently identified. Sentinel birds should be used only if no appropriate *laboratory* procedures are available. The interpretation of serological results in the presence of *vaccination* is described in Article 10.4.33.

Article 10.4.30.

#### Surveillance for demonstrating Documentation of freedom from avian influenza or freedom from infection with high pathogenicity avian influenza viruses in poultry

##### 1. Additional surveillance requirements for Member Countries declaring freedom of the country, zone or compartment from avian influenza or from infection with high pathogenicity avian influenza viruses in poultry

In addition to the general conditions described in above mentioned articles, a A Member Country declaring freedom of the entire country, or a *zone* or a *compartment* from avian influenza or from *infection* with high pathogenicity avian influenza viruses in *poultry* should provide evidence for the existence of an effective *surveillance* programme.

The strategy and design of the *surveillance* programme depend on the prevailing epidemiological circumstances and should be planned and implemented according to general conditions and methods described in this chapter and in Article 1.4.6, to demonstrate absence of *infection* with avian influenza viruses or with high pathogenicity avian influenza viruses, during the preceding 12 months in susceptible *poultry* populations (vaccinated and non-vaccinated). This requires the availability of demographic data on the poultry population and the support of a laboratory able to undertake identification of infection with avian influenza viruses through virus detection and antibody tests. This *surveillance* may be targeted to *poultry* population at specific risks linked to the types of production, possible direct or indirect contact with *wild* birds, multi-age *flocks*, local trade patterns including live bird markets, use of possibly contaminated surface water, and the presence of more than one species on the holding establishment and poor *biosecurity* measures in place. It should include the monitoring of high pathogenicity avian influenza virus in wild birds and of H5 and H7 low pathogenicity avian influenza virus in poultry, in order to adapt the biosecurity and possible control measures.

Documentation for freedom from infection with high pathogenicity avian influenza should provide details of the poultry population, the occurrence of suspected cases and how they were investigated and dealt with. This should include the results of laboratory testing and the biosecurity and control measures to which the animals concerned were subjected during the investigation.

##### 2. Additional requirements for countries, zones or compartments that practice vaccination

*Vaccination* to prevent the transmission of high pathogenicity avian influenza virus may be part of a *disease* control programme. The level of *flock* immunity required to prevent transmission depends on the *flock* size, composition (e.g. species) and density of the susceptible *poultry* population. It is therefore impossible to be prescriptive. Based on the epidemiology of avian influenza in the country, *zone* or *compartment*, it may be that a decision is reached to vaccinate only certain species or other *poultry* subpopulations.

Annex 19 (contd)

In all vaccinated *flocks* there is a need to perform virological and serological tests to ensure the absence of virus circulation. The use of sentinel *poultry* may provide further confidence of the absence of virus circulation. The tests have to be repeated at least every six months or at shorter intervals according to the *risk* in the country, *zone* or *compartment*.

Evidence to show the effectiveness of the *vaccination* programme should also be provided.

Member Countries seeking the demonstration of freedom from high pathogenicity avian influenza in vaccinated population should refer to Chapter 2.3.4. paragraph C 4 of the *Terrestrial Manual*.

### 3. Additional requirements for recovery of free status

In addition to the conditions described in the point above, a Member Country declaring that it has regained country, zone or compartment freedom after an outbreak of high pathogenicity avian influenza in poultry should show evidence of an active surveillance programme depending on the epidemiological circumstances of the outbreak to demonstrate the absence of the infection. This will require surveillance incorporating virus detection and antibody tests. The use of sentinel birds may facilitate the interpretation of surveillance results. The Member Country should report the results of an active surveillance programme in which the susceptible poultry population undergoes regular clinical examination and active surveillance planned and implemented according to the general conditions and methods described in these recommendations. The surveillance samples should be representative of poultry populations at risk.

Populations under this surveillance programme should include:

- 1) establishments in the proximity of the outbreaks;
- 2) establishments epidemiologically linked to the outbreaks;
- 3) animals moved from or used to re-populate affected establishments;
- 4) any establishments where contiguous culling has been carried out;

Article 10.4.30bis.

### Surveillance of wild bird populations

The presence of high pathogenicity avian influenza viruses in wild birds creates a particular problem. In essence, no Member Country can declare itself free from influenza A viruses in wild birds. However, the definition of high pathogenicity avian influenza in this chapter refers to the infection in poultry only, and Articles 10.4.27. to 10.4.33. were developed under this definition.

Passive surveillance (i.e. sampling of birds found dead) is an appropriate method of surveillance in wild birds as infection with high pathogenicity avian influenza is usually associated with mortality. Mortality events, or clusters of birds found dead should be reported to the local Veterinary Authorities and investigated.

Active surveillance in wild birds usually has lower sensitivity for detection of high pathogenicity avian influenza, but may be necessary for detection of some strains of high pathogenicity avian influenza virus that produce infection without mortality in wild birds.

Surveillance in wild birds should be targeted towards species, locations and times of year in which infection is more likely.

Surveillance in wild birds should be enhanced by awareness raising and active searching and monitoring for dead or moribund wild birds when high pathogenicity avian influenza has been detected in the region. The movements of migratory water birds, in particular ducks, geese and swans, should be taken into account as a potential pathway for introduction of virus to uninfected areas.

Article 10.4.30ter.Monitoring of H5 and H7 low pathogenicity avian influenza in poultry populations

Monitoring the presence of H5 and H7 low pathogenicity avian influenza viruses can be achieved through the combination of clinical investigations where infection is suspected through changes in production indicators such as reductions in egg production or feed and water intake and active serological and virological surveillance.

Serological monitoring should aim at detecting clusters of infected flocks to identify spread between establishments. Epidemiological follow-up (tracing forward and back) of serologically positive flocks should be carried out to determine if there is clustering of infected flocks regardless of whether the seropositive birds are still present on the establishment or whether active virus infection has been detected.

~~Article 10.4.31.~~~~Additional surveillance requirements for countries, zones or compartments declaring that they have regained freedom from avian influenza or from infection with high pathogenicity avian influenza viruses in poultry following an outbreak~~

~~In addition to the general conditions described in the above-mentioned articles, a Member Country declaring that it has regained country, zone or compartment freedom from avian influenza or from infection with high pathogenicity avian influenza viruses in poultry should show evidence of an active surveillance programme depending on the epidemiological circumstances of the outbreak to demonstrate the absence of the infection. This will require surveillance incorporating virus detection and antibody tests. The use of sentinel birds may facilitate the interpretation of surveillance results.~~

~~A Member Country declaring freedom of country, zone or compartment after an outbreak of avian influenza should report the results of an active surveillance programme in which the susceptible poultry population undergoes regular clinical examination and active surveillance planned and implemented according to the general conditions and methods described in these recommendations. The surveillance should at least give the confidence that can be given by a randomised representative sample of the populations at risk.~~

~~Article 10.4.32.~~~~Additional surveillance requirements for the avian influenza free establishments~~

~~The declaration of avian influenza free establishments requires the demonstration of absence of infection with avian influenza viruses. Birds in these establishments should be randomly tested using virus detection or isolation tests, and serological methods, following the general conditions of these recommendations. The frequency of testing should be based on the risk of infection and at a maximum interval of 24 28 days.~~

~~Article 10.4.33.~~~~The use and interpretation of serological and virus detection tests~~

~~Poultry infected with avian influenza virus produce antibodies against haemagglutinin (HA), neuraminidase (NA), nonstructural proteins (NSPs), nucleoprotein/matrix (NP/M) and the polymerase complex proteins. Detection of antibodies against the polymerase complex proteins is not covered in this chapter. Tests for NP/M antibodies include direct and blocking ELISA, and agar gel immunodiffusion (AGID) tests. Tests for antibodies against NA include the neuraminidase inhibition (NI), indirect fluorescent antibody and direct and blocking ELISA tests. For the HA, antibodies are detected in haemagglutination inhibition (HI), ELISA and neutralisation (SN) tests. The HI test is reliable in avian species but not in mammals. The SN test can be used to detect subtype-specific antibodies against the haemagglutinin and is the preferred test for mammals and some avian species. The AGID test is reliable for detection of NP/M antibodies in chickens and turkeys, but not in other avian species. As an alternative, blocking ELISA tests have been developed to detect NP/M antibodies in all avian species.~~

~~The HI and NI tests can be used to subtype influenza A viruses into 16 haemagglutinin and 9 neuraminidase subtypes. Such information is helpful for epidemiological investigations and in categorization of influenza A viruses.~~

Annex 19 (contd)

~~Poultry can be vaccinated with a variety of influenza A vaccines including inactivated whole virus vaccines, and haemagglutinin expression-based vaccines. Antibodies against the haemagglutinin confer subtype specific protection. Various strategies can be used to differentiate vaccinated from infected birds including serosurveillance in unvaccinated sentinel birds or specific serological tests in the vaccinated birds.~~

~~Influenza A virus infection of unvaccinated birds including sentinels is detected by antibodies against the NP/M, subtype specific HA or NA proteins, or NSP. Poultry vaccinated with inactivated whole virus vaccines containing a virus of the same H sub-type but with a different neuraminidase may be tested for field exposure by applying serological tests directed to the detection of antibodies against the NA of the field virus. For example, birds vaccinated with H7N3 in the face of a H7N1 epidemic may be differentiated from infected birds (DIVA) by detection of subtype specific NA antibodies of the N1 protein of the field virus. Alternatively, in the absence of DIVA, inactivated vaccines may induce low titres of antibodies against NSP and the titre in infected birds would be markedly higher. Encouraging results have been obtained experimentally with this system, but it has not yet been validated in the field. In poultry vaccinated with haemagglutinin expression-based vaccines, antibodies are detected against the specific HA, but not any of the other viral proteins. Infection is evident by antibodies against the NP/M or NSP, or the specific NA protein of the field virus.~~

~~All flocks with seropositive results should be investigated. Epidemiological and supplementary laboratory investigation results should document the status of avian influenza infection for each positive flock.~~

~~A confirmatory test should have a higher specificity than the screening test and sensitivity at least equivalent than that of the screening test.~~

~~Information should be provided on the performance characteristics and validation of tests used.~~

#### ~~4. Procedure in case of positive test results if vaccination is used~~

~~In case of vaccinated populations, one has to exclude the likelihood that positive test results are indicative of virus circulation. To this end, the following procedure should be followed in the investigation of positive serological test results derived from surveillance conducted on vaccinated poultry. The investigation should examine all evidence that might confirm or refute the hypothesis that the positive results to the serological tests employed in the initial survey were not due to virus circulation. All the epidemiological information should be substantiated, and the results should be collated in the final report.~~

~~Knowledge of the type of vaccine used is crucial in developing a serological based strategy to differentiate infected from vaccinated animals.~~

- ~~a) Inactivated whole virus vaccines can use either homologous or heterologous neuraminidase subtypes between the vaccine and field strains. If poultry in the population have antibodies against NP/M and were vaccinated with inactivated whole virus vaccine, the following strategies should be applied:
 
  - ~~i) sentinel birds should remain NP/M antibody negative. If positive for NP/M antibodies, indicating influenza A virus infection, specific HI tests should be performed to identify H5 or H7 virus infection;~~
  - ~~ii) if vaccinated with inactivated whole virus vaccine containing homologous NA to field virus, the presence of antibodies against NSP could be indicative of infection. Sampling should be initiated to exclude the presence of avian influenza virus by either virus isolation or detection of virus specific genomic material or proteins;~~
  - ~~iii) if vaccinated with inactivated whole virus vaccine containing heterologous NA to field virus, presence of antibodies against the field virus NA or NSP would be indicative of infection. Sampling should be initiated to exclude the presence of avian influenza virus by either virus isolation or detection of virus specific genomic material or proteins.~~~~
- ~~b) Haemagglutinin expression-based vaccines contain the HA protein or gene homologous to the HA of the field virus. Sentinel birds as described above can be used to detect avian influenza infection. In vaccinated or sentinel birds, the presence of antibodies against NP/M, NSP or field virus NA is indicative of infection. Sampling should be initiated to exclude the presence of avian influenza virus by either virus isolation or detection of virus specific genomic material or proteins.~~

## 2- Procedure in case of test results indicative of infection with avian influenza viruses

The detection of antibodies indicative of an *infection* with avian influenza virus in unvaccinated *poultry* should result in the initiation of epidemiological and virological investigations to determine if the *infections* are due to low and high pathogenicity viruses.

Virological testing should be initiated in all antibody positive and at risk populations. The samples should be evaluated for the presence of avian influenza virus, by virus isolation and identification, or detection of influenza A specific proteins or nucleic acids (Figure 2). Virus isolation is the gold standard for detecting *infection* by avian influenza virus. All influenza A virus isolates should be tested to determine HA and NA subtypes, and *in vivo* tested in chickens or sequencing of HA proteolytic cleavage site of H5 and H7 subtypes for determination of classification as high or low pathogenicity avian influenza viruses or other influenza A viruses. As an alternative, nucleic acid detection tests have been developed and validated; these tests have the sensitivity of virus isolation, but with the advantage of providing results within a few hours. Samples with detection of H5 and H7 HA subtypes by nucleic acid detection methods should either be submitted for virus isolation, identification, and *in vivo* testing in chickens, or sequencing of nucleic acids for determination of proteolytic cleavage site as high or low pathogenicity avian influenza viruses. The use of antigen detection systems, because of low sensitivity, should be limited to screening clinical field cases for *infection* by influenza A virus looking for NP/M proteins. NP/M positive samples should be submitted for virus isolation, identification and pathogenicity determination.

Laboratory results should be examined in the context of the epidemiological situation. Corollary information needed to complement the serological survey and assess the possibility of viral circulation includes but is not limited to:

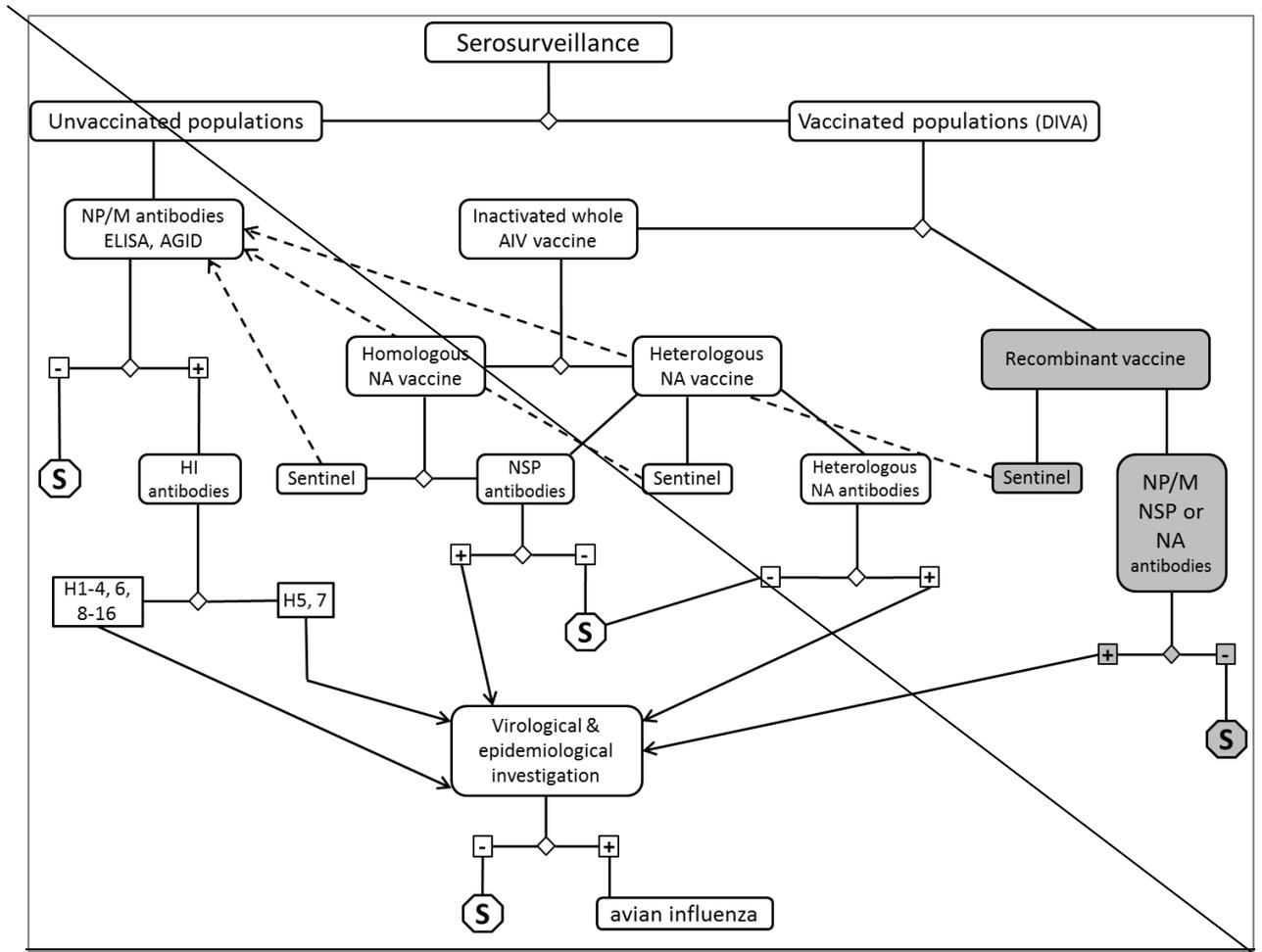
- a) characterisation of the existing production systems;
- b) results of clinical *surveillance* of the suspects and their cohorts;
- c) quantification of *vaccinations* performed on the affected sites;
- d) sanitary protocol and history of the affected *establishments*;
- e) control of animal identification and movements;
- f) other parameters of regional significance in historic avian influenza virus transmission.

The entire investigative process should be documented as standard operating procedure within the epidemiological *surveillance* programme.

Figures 1 and 2 indicate the tests which are recommended for use in the investigation of *poultry flocks*.

Key abbreviations and acronyms:	
AGID	Agar gel immunodiffusion
DIVA	Differentiating infected from vaccinated animals
ELISA	Enzyme-linked immunosorbent assay
HA	Haemagglutinin
HI	Haemagglutination inhibition
NA	Neuraminidase
NP/M	Nucleoprotein and matrix protein
NSP	Nonstructural protein
S	No evidence of avian influenza virus

**Fig. 1.** Schematic representation of laboratory tests for determining evidence of avian influenza infection through or following serological surveys



**Fig. 2.** Schematic representation of laboratory tests for determining evidence of avian influenza infection using virological methods

