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Ottawa, ONT
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Action T-294-25

FEDERAL COURT

BETWEEN

UNIVERSAL OSTRICH FARMS INC.

APPLICANT

AND:

CANADIAN FOOD INSPECTION AGENCY

RESONDENT

AFFIDAVIT

I, KATRINA JONES, Legal Assistant, of 1321 Johnston Street, White Rock, British Columbia, MAKE OATH AND SWEAR THAT:

1. I am a legal assistant to Michael D. Carter, the lawyer for the Applicant in this matter, and as such have personal knowledge of the facts and matters hereinafter deposed to, save and except where the same are stated to be based upon information or belief and where so stated I verily believe such statements to be true.

2. On January 30, 2025, I found the World Organisation for Animal Health (WOAH) objectives on the Government of Canada (GOC) website. Attached hereto and marked as **Exhibit "A"** to this Affidavit is a copy of the GOC website page, which speaks to WOAH's objectives.

3. On January 30, 2025 I found WOAH's Terrestrial Animal Health Code (2024) (the "WOAH Health Code") on the WOAH webpage. Attached hereto and marked as **Exhibit "B"** to this Affidavit is a copy of the specific sections of the WOAH Health Code that speaks to the high pathogenicity avian influenza viruses, its glossary, and WHOA's obligations.

4. On January 30, 2025 I found WOAH's Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (the "WOAH Manual") on the WOAH webpage. Attached hereto and marked as **Exhibit "C"** to this Affidavit is a copy of the specific

section of the WOAH Manual that addresses high pathogenicity avian influenza viruses.

5. On January 29, 2025, I found CFIA's information page regarding how it prevents, prepares and responds to bird flu outbreaks. Attached hereto and marked as **Exhibit "D"** to this Affidavit is a copy of the CFIA webpage that addresses its response and prevention of the bird flu.

6. On January 28, 2025, I found CFIA's information page regarding its Highly Pathogenic Avian Influenza Vaccination Task Force. Attached hereto and marked as **Exhibit "E"** to this Affidavit is a copy of the CFIA webpage that speaks to the Vaccination Task Force.

7. On January 30, 2025, I found CFIA's information page regarding avian influenza immunity and vaccination. Attached hereto and marked as **Exhibit "F"** to this Affidavit is a copy of the CFIA webpage that speaks to avian influenza immunity and vaccination.

8. On January 28, 2025, I found CFIA's Open and Transparent Agent Policy. Attached hereto and marked as **Exhibit "G"** to this Affidavit is a copy of the CFIA's Open and Transparent Agent Policy from its webpage.

9. On January 30, 2025, I found CFIA's Policy for Providing Guidance on Regulatory Requirements. Attached hereto and marked as **Exhibit "H"** to this Affidavit is a copy of the CFIA's Policy for Providing Guidance on Regulatory Requirements from its webpage.

10. On January 30, 2025, I found the CFIA's Field-ready lateral flow test for avian influenza, wherein the CFIA states that the National Centre for Foreign Animal Disease (NCFAD) is located in Winnipeg, which is a WOAH reference laboratory for the avian influenza. Attached hereto and marked as **Exhibit "I"** to this Affidavit is a copy of the CFIA's webpage.

11. On January 30, 2025, I found the CFIA's latest bird flu situation updates. Attached hereto and marked as **Exhibit "J"** to this Affidavit is a copy of the CFIA's webpage.

12. On January 29, 2025, I conducted an online search of the distance it would take to drive from Castlegar, British Columbia to Edgewood, BC V0G 1J0 on Google Maps. Attached hereto and marked as **Exhibit "K"** to this Affidavit is a copy of the Google Maps route.

13. On January 29, 2025, I conducted an online search of the distance it would take to drive from Vernon, British Columbia to Edgewood, BC V0G 1J0 on Google

Maps. Attached hereto and marked as **Exhibit "L"** to this Affidavit is a copy of the Google Maps route.

14. Attached hereto and marked as **Exhibit "M"** is a true copy a sales invoice received from Universal Ostrich Farms Inc.

SWORN (OR AFFIRMED) BEFORE ME at White Rock, British Columbia on January 30, 2025

.....

A commissioner for taking affidavits for British Columbia

MICHAEL D. CARTER
Barrister & Solicitor
1321 Johnston Road
White Rock, B.C. V4B 3Z3
(604) 536-5002

KATRINA JONES

World Organisation for Animal Health (WOAH)

The World Organisation for Animal Health (WOAH; founded as Office International des Épizooties (OIE)) is the science based standard setting organization at the international level for animal and veterinary public health. It also serves as the scientific reference body for international trade of animals and animal derived products under the Sanitary and Phyto-sanitary (SPS) Agreement of the World Trade Organization.

The WOAH (World Organization for Animal Health)'s objectives are:

- To ensure transparency in the global animal disease and zoonosis situation
- To collect, analyse and disseminate scientific veterinary information
- To provide expertise and encourage international solidarity in the control of animal diseases
- Within its mandate under the WTO (World Trade Organization) SPS (Sanitary and Phyto-sanitary) Agreement, to safeguard world trade by publishing health standards for international trade in animals and animal products
- To improve the legal framework and resources of National Veterinary Services
- To provide a better guarantee of the safety of food of animal origin and to promote animal welfare through a science-based approach

The duties of the WOAH (World Organisation for Animal Health) Delegate for Canada include, but are not limited to:

- Representing Canada at the World Assembly of Delegates and voting on international standards, recommendations, and resolutions
- Notifying the WOAH (World Organisation for Animal Health) of animal diseases present in Canada
- Bringing the resolutions of the World Assembly to the attention of the Canadian government, and ensuring that, as far as possible, the resolution of the World Assembly are applied in Canada
- Providing scientific input into the development of international standards, and
- Designating national focal points for support in the fields of animal health information, wildlife diseases, veterinary medicinal products, animal production food safety, animal welfare, communications and laboratories

This is Exhibit "A" referred to in the affidavit of Katrina Jones sworn before me at Whitehorse this 30 day of January 20 25

International Standards

International Sanitary Standards are drafted by the WOAH (World Organization for Animal Health) Specialist Commissions. Standards are created to protect countries from the introduction of diseases and pathogens, while ensuring they are fair and scientifically justified. These sanitary standards are continually revised and updated.

At the Specialist Commission level comments that are supported by sound scientific information will be taken into account and draft standards may be revised accordingly. All revised draft standards are submitted to the WOAH (World Organization for Animal Health) for ratification by the International Committee at the General Session. Ratified standards are then incorporated into the relevant WOAH (World Organization for Animal Health) publications.

- Manual of Diagnostic Test and Vaccines for Terrestrial Animals
- Manual of Diagnostic Tests for Aquatic Animals
- Terrestrial Animal Health Code
- Aquatic Animal Health Code

The WOAH (World Organization for Animal Health) oversees four specialist commissions that develop and revise the WOAH's international sanitary standards, by addressing scientific and technical issues raised by Member Countries.

- Terrestrial Animal Health Standards Commission ("Code Commission") - establishes standards governing the trade of terrestrial animals and animal products.
- Scientific Commission for Animal Diseases ("Scientific Commission")- assists in identifying the most appropriate strategies and measures for disease prevention and control. The Commission also reviews submissions regarding animal health status for Member Countries that wish to be included on the WOAH's list of countries 'free' of certain diseases.
- Biological Standards Commission ("Laboratories Commission")- establishes methods for diagnosing diseases of mammals, birds and bees. Furthermore, the Commission tests biological products, such as vaccines. It oversees the production of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.
- Aquatic Animal Health Standards Commission ("Aquatic Commission")- compiles information on diseases of fish, molluscs and crustaceans, and on methods used to control these diseases.

In addition to the four Specialist Commissions, there are three working groups focusing on Wildlife Diseases, Animal Welfare and Food Safety. The purpose of the working groups is to collect, analyse and disseminate information relevant to their respective fields.

WOAH's Performance of Veterinary Services (PVS) Evaluation Report of Canada

The WOAH has evaluated Canada's veterinary services and has found Canada to be a top performing country and a leading example for meeting international veterinary service standards. The report is now available on the WOAH's website.

- WOAH's PVS evaluation report of Canada's veterinary services
- Notice to industry: WOAH releases PVS report on its evaluation of Canada's veterinary services
- CVO Statement: WOAH's PVS evaluation report of Canada (2018-08-02)
- Infographic: How Canada's veterinary services measure up in the world

Date modified:
2018-11-05

CHAPTER 10.4.

INFECTION WITH HIGH PATHOGENICITY AVIAN INFLUENZA VIRUSES

Article 10.4.1.

General provisions

1) This chapter deals with the *listed disease*, *infection* with 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 that has been determined as high pathogenicity in accordance with the *Terrestrial Manual*.

   b) An occurrence of *infection* with a high pathogenicity avian influenza virus is defined by the isolation and identification of the virus or the detection of specific viral ribonucleic acid, in one or more samples from *poultry*.

   c) The *incubation period* at the *flock*-level for high pathogenicity avian influenza is 14 days.

3) Although the objective of this chapter is to mitigate animal and public health risks posed by *infection* with high pathogenicity avian influenza viruses, other influenza A viruses of avian host origin (i.e. low pathogenicity avian influenza viruses) may have the potential to exert a negative impact on animal and public health. A sudden and unexpected increase in virulence of low pathogenicity avian influenza viruses in *poultry* is notifiable as an *emerging disease* in accordance with Article 1.1.4. *Infection* of domestic and *captive wild* birds with low pathogenicity avian influenza viruses having proven natural transmission to humans associated with severe consequences, and *infection* of birds other than *poultry*, including *wild* birds, with influenza A viruses of high pathogenicity, are notifiable in accordance with Article 1.3.3.

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

5) This chapter includes *monitoring* considerations for low pathogenicity avian influenza viruses because some, especially H5 and H7 subtypes, have the potential to mutate into high pathogenicity avian influenza viruses.

6) The use of *vaccination* against avian influenza may be recommended under specific conditions. Any vaccine used should comply with the standards described in the *Terrestrial Manual*. *Vaccination* will not affect the high pathogenicity avian influenza status of a free country or *zone* if *surveillance* supports the absence of *infection*, in accordance with Article 10.4.28., in particular point 2. *Vaccination* can be used as an effective complementary control tool when a *stamping-out policy* alone is not sufficient. Whether to vaccinate or not should be decided by the *Veterinary Authority* on the basis of the avian influenza situation as well as the ability of the *Veterinary Services* to implement the *vaccination* strategy, as described in Chapter 4.18.

7) Standards for diagnostic tests and vaccines, including pathogenicity testing, are described in the *Terrestrial Manual*.

Article 10.4.2.

Safe commodities

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

1) heat-treated *poultry meat products* in a hermetically sealed container with an F_0 value of 3 or above;

2) extruded dry pet food and coated ingredients after extrusion;

3) rendered *protein meal*, blood meal, feather meal, and *poultry* oil;

4) washed and steam-dried feathers and down from *poultry* and other birds.

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

Article 10.4.3.

Country or zone free from high pathogenicity avian influenza

A country or *zone* may be considered free from high pathogenicity avian influenza when:

- *infection* with high pathogenicity avian influenza viruses is a *notifiable disease* in the entire country;
- an ongoing awareness programme is in place to encourage reporting of suspicions of high pathogenicity avian influenza;
- absence of *infection* with high pathogenicity avian influenza viruses, based on *surveillance*, in accordance with Chapter 1.4. and Articles 10.4.26. to 10.4.30., has been demonstrated in the country or *zone* for the past 12 months;
- an awareness programme is in place related to avian influenza viruses *risks* and the specific *biosecurity* and management measures to address them;
- *commodities* are imported in accordance with Articles 10.4.7. to 10.4.22.

Surveillance should be adapted to parts of the country or existing *zones* depending on historical or geographical factors, industry structure, population data and proximity to recent *outbreaks* or the use of *vaccination*.

Article 10.4.4.

Compartment free from high pathogenicity avian influenza

The establishment of a *compartment* free from high pathogenicity avian influenza should be in accordance with relevant requirements of this chapter and the principles described in Chapters 4.4. and 4.5.

Article 10.4.5.

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 purpose 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 4.4.7., the *surveillance* programme should take into account the density of *poultry* production, types of *poultry*, local management practices (including inter-premises movement patterns of *poultry*, people and equipment), relevant *biosecurity*, the presence and potential role of birds other than *poultry*, including *wild* birds, and the proximity of *poultry establishments* to permanent 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 10.4.6., once the *containment zone* is established. It should be demonstrated that *commodities* for *international trade* have originated from outside the *containment zone* or comply with the relevant articles of this chapter.

Article 10.4.6.

Recovery of free status

If *infection* with high pathogenicity avian influenza virus has occurred in *poultry* in a previously free country or *zone*, the free status may be regained after a minimum period of 28 days (i.e. two *flock*-level *incubation periods*) after a *stamping-out policy* has been completed (i.e. after the *disinfection* of the last affected *establishment*), provided that *surveillance* in accordance with Articles 10.4.26. to 10.4.30., in particular point 3 of Article 10.4.28., 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 10.4.7.

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) the *poultry* originated from a country, *zone* or *compartment* free from high pathogenicity avian influenza;
3) the *poultry* originated from a *flock* that was monitored for avian influenza viruses and was found to be negative;
4) the *poultry* are transported in new or appropriately sanitised *containers*.

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

Article 10.4.8.

Recommendations for the importation of live birds other than poultry

Regardless of the high pathogenicity 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 avian influenza;
2) the birds had been kept in isolation facilities approved by the *Veterinary Services* since they were hatched or for at least 28 days (i.e. two *flock*-level *incubation periods*) prior to shipment and showed no clinical signs of avian influenza during the isolation period;
3) a statistically appropriate sample of the birds was subjected, with negative results, to a diagnostic test for avian influenza within 14 days prior to shipment;
4) the birds are transported in new or appropriately sanitised *containers*.

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

Article 10.4.9.

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

For day-old live poultry

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

1) the day-old live *poultry* had been kept in a country, *zone* or *compartment* free from high pathogenicity avian influenza since they were hatched;

   and

   a) the day-old live *poultry* were derived from parent *flocks* that were monitored for avian influenza viruses and were found to be negative at the time of collection of the eggs from which the day-old *poultry* hatched; or
   b) the day-old live *poultry* that hatched from eggs that had had their surfaces sanitised in accordance with point 4 d) of Article 6.5.5.;

AND

2) the day-old live *poultry* were transported in new or appropriately sanitised *containers*.

If the day-old live *poultry* or the parent *flocks* have been vaccinated against avian influenza, the nature of the vaccine used and the date of *vaccination* should be stated in the *international veterinary certificate*.

Article 10.4.10.

Recommendations for the importation of day-old live birds other than poultry

Regardless of the high pathogenicity 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 avian influenza;
2) the birds were hatched and kept in isolation facilities approved by the *Veterinary Services*;
3) a statistically appropriate sample of the parent *flock* birds were subjected, with negative results, to a diagnostic test for avian influenza at the time of collection of the eggs;
4) the birds were transported in new or appropriately sanitised *containers*.

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 stated in the *international veterinary certificate*.

Article 10.4.11.

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

For hatching eggs of poultry

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

1) the hatching eggs came from a country, *zone* or *compartment* free from high pathogenicity avian influenza;
2)
   a) the hatching eggs were derived from parent *flocks* that were monitored for avian influenza viruses and were found to be negative at the time of collection of the hatching eggs; or
   b) the hatching eggs have had their surfaces sanitised in accordance with point 4 d) of Article 6.5.5.;
3) the hatching eggs are transported in new or appropriately sanitised packaging materials and *containers*.

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

Article 10.4.12.

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

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

1) a statistically appropriate sample of the parent *flock* birds was subjected, with negative results, to a diagnostic test for avian influenza 14 days prior to and at the time of collection of the hatching eggs;
2) the hatching eggs have had their surfaces sanitised in accordance with point 4 d) of Article 6.5.5.;
3) the hatching eggs are transported in new or appropriately sanitised packaging materials and *containers*.

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

Article 10.4.13.

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

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 avian influenza on the day of semen collection;

2) were kept in a country, *zone* or *compartment* free from high pathogenicity avian influenza.

Article 10.4.14.

Recommendations for the importation of semen from birds other than poultry

Regardless of the high pathogenicity 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 facilities approved by the *Veterinary Services* for at least 28 days (i.e. two *flock*-level *incubation periods*) prior to semen collection;
2) showed no clinical signs of avian influenza during the isolation period;
3) were subjected, with negative results, to a diagnostic test for avian influenza within 14 days prior to semen collection.

Article 10.4.15.

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

For eggs for human consumption

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

1) the eggs for human consumption were produced and packed in a country, *zone* or *compartment* free from high pathogenicity avian influenza;
2) the eggs for human consumption were transported in new or appropriately sanitised packaging materials and *containers*.

Article 10.4.16.

Recommendations for the importation of egg products from poultry

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

1) the egg products are derived from eggs which meet the requirements of Article 10.4.15.; or
2) the egg products have been processed to ensure the inactivation of high pathogenicity avian influenza viruses, in accordance with Article 10.4.23.;

AND

3) the necessary precautions were taken to avoid contact of the egg products with any source of high pathogenicity avian influenza viruses.

Article 10.4.17.

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

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 originated from a country, *zone* or *compartment* free from high pathogenicity avian influenza;
2) which were slaughtered in an approved *slaughterhouse/abattoir* in a country, *zone* or *compartment* free from high pathogenicity avian influenza and were subjected to ante- and post-mortem inspections in accordance with Chapter 6.3., with favourable results.

Article 10.4.18.

Recommendations for the importation of meat products from poultry

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

1) the *meat products* from *poultry* are derived from *fresh meat* which meets the requirements of Article 10.4.17.; or

2) the *meat products* from *poultry* have been processed to ensure the inactivation of high pathogenicity avian influenza viruses in accordance with Article 10.4.24.;

AND

3) the necessary precautions were taken to avoid contact of the *meat products* from *poultry* with any source of high pathogenicity avian influenza viruses.

Article 10.4.19.

Recommendations for the importation of poultry products not listed in Article 10.4.2. and intended for use in animal feeding, or for agricultural or industrial use

Regardless of the high pathogenicity 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 obtained from *poultry* which originated in a country, *zone* or *compartment* free from high pathogenicity avian influenza and that the necessary precautions were taken to avoid contamination during processing with any source of high pathogenicity avian influenza viruses;

OR

2) these *commodities* have been processed to ensure the inactivation of high pathogenicity avian influenza viruses using:

   a) moist heat treatment for 30 minutes at 56°C; or

   b) heat treatment where the internal temperature throughout the product reached at least 74°C; or

   c) any equivalent treatment that has been demonstrated to inactivate avian influenza viruses;

AND

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

Article 10.4.20.

Recommendations for the importation of feathers and down from poultry not listed in Article 10.4.2.

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

1) these *commodities* originated from *poultry* as described in Article 10.4.17. and were processed 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 viruses using one of the following:

   a) fumigation with formalin (10% formaldehyde) for 8 hours;

   b) irradiation with a dose of 20 kGy;

   c) any equivalent treatment which has been demonstrated to inactivate avian influenza viruses;

AND

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

Article 10.4.21.

Recommendations for the importation of feathers and down of birds other than poultry not listed in Article 10.4.2.

Regardless of the high pathogenicity 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 inactivation of high pathogenicity avian influenza viruses using one of the following:
   a) fumigation with formalin (10% formaldehyde) for 8 hours;
   b) irradiation with a dose of 20 kGy;
   c) any equivalent treatment which has been demonstrated to inactivate avian influenza viruses;
2) the necessary precautions were taken to avoid contact of the *commodity* with any source of high pathogenicity avian influenza viruses.

Article 10.4.22.

Recommendations for the importation of collection specimens, skins and trophies of birds other than poultry

Regardless of the high pathogenicity 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 inactivation of high pathogenicity avian influenza viruses in accordance with Article 10.4.25.;

AND

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

Article 10.4.23.

Procedures for the inactivation of high pathogenicity avian influenza viruses in egg products from poultry

The following time/temperature combinations are suitable for the inactivation of high pathogenicity avian influenza viruses present in 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

These time/temperature combinations are indicative of a range that achieves a 7-$\log_{10}$ reduction of avian influenza virus infectivity. These are examples for a variety of egg products but, when supported by scientific evidence, variations of these time/temperature combinations may be used, and they may be used for other egg products, if they achieve equivalent inactivation of the virus.

Article 10.4.24.

Procedures for the inactivation of high pathogenicity avian influenza viruses in meat products from poultry

The following time/temperature combinations are suitable for the inactivation of high pathogenicity avian influenza viruses in *meat products*.

	Core temperature (°C)	Time
Meat products from poultry	60.0	507 seconds
	65.0	42 seconds
	70.0	3.5 seconds
	73.9	0.51 second

These time/temperature combinations are indicative of a range that achieves a 7-$\log_{10}$ reduction of avian influenza virus infectivity. When supported by scientific evidence, variations of these time/temperature combinations may be used if they achieve equivalent inactivation of the virus.

Article 10.4.25.

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

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

1) boiling in water for an appropriate time to ensure that any material 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_2CO_3) 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, treatment for at least 28 days with salt (NaCl) containing 2% washing soda (sodium carbonate-Na_2CO_3); or
5) treatment with 1% formalin for a minimum of six days; or
6) any equivalent treatment which has been demonstrated to inactivate the virus.

Article 10.4.26.

Principles of surveillance for avian influenza

The following are complementary to Chapter 1.4. and should be applied by Member Countries seeking to determine their high pathogenicity avian influenza status.

These principles are also necessary to support *vaccination* programmes, to monitor low pathogenicity avian influenza viruses, especially H5 and H7, in *poultry* and to detect high pathogenicity avian influenza in *wild* birds.

The impact and epidemiology of avian influenza differ widely among different regions of the world and therefore it is impossible to provide detailed recommendations for all situations. Variables such as the frequency of contacts between *poultry* and *wild* birds, different *biosecurity* levels and production systems, and the commingling of different susceptible species including domestic waterfowl, may require different *surveillance* strategies to address each situation. Furthermore, domestic waterfowl typically do not show clinical signs and have longer infective periods than gallinaceous *poultry*. It is therefore incumbent upon the Member Country to provide scientific data that explain the epidemiology of avian influenza in the region of concern and also to demonstrate how all the risk factors have been taken into account. Member Countries have flexibility to provide a science-based approach to demonstrate absence of *infection* with high pathogenicity avian influenza viruses at an appropriate level of confidence, as described in Chapter 1.4.

There is an increased recognition of the value of the application of sequencing technologies and phylogenetic analyses to determine routes of introduction, transmission pathways and epidemiological patterns of *infection*. When avian influenza viruses are detected, Member Countries should apply these technologies, when possible, to enhance the evidence used to develop specific *surveillance* strategies and control activities.

A *monitoring* system for low pathogenicity avian influenza viruses in *poultry* should be in place for the following reasons:

1) H5 and H7 low pathogenicity avian influenza viruses have the potential to mutate into high pathogenicity avian influenza viruses, but it is not possible to predict which viruses will mutate or when these mutations will occur.
2) The detection of sudden and unexpected increases in virulence of low pathogenicity avian influenza viruses in *poultry* is notifiable as an *emerging disease* in accordance with Article 1.1.4.
3) The detection, in domestic or *captive wild* birds, of low pathogenicity avian influenza viruses that have been proven to be transmitted naturally to humans with severe consequences is notifiable in accordance with Article 1.1.3.

Article 10.4.27.

Surveillance for early warning of high pathogenicity avian influenza

1) An ongoing *surveillance* programme for avian influenza should be in place and be designed to detect the presence of *infection* with high pathogenicity avian influenza viruses in the country or *zone* in a timely manner.
2) The high pathogenicity avian influenza *surveillance* programme should include the following.
   a) An *early warning system* for reporting suspected *cases*, in accordance with Article 1.4.5. throughout the production, marketing and processing chain. Farmers and workers who have day-to-day contact with *poultry*, as well as diagnosticians, should report promptly any suspicion of avian influenza to the *Veterinary Services*. All suspected *cases* of high pathogenicity avian influenza should be investigated immediately and samples should be collected and submitted to a *laboratory* for appropriate tests.
   b) Implementation, as relevant, of regular and frequent clinical inspection, or serological and virological testing of high-risk groups of *animals*, such as those adjacent to a country or *zone* infected with high pathogenicity avian influenza, places where birds and *poultry* of different origins are mixed, such as live bird markets, and *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) Immediate investigation of the presence of antibodies against influenza A viruses that have been detected in *poultry* and are not a consequence of *vaccination*. In the case of single or 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*.

Article 10.4.28.

Surveillance for demonstrating freedom from infection with high pathogenicity avian influenza

1) A Member Country declaring freedom of the entire country, a *zone* or a *compartment* from high pathogenicity avian influenza in *poultry* should provide evidence of an effective *surveillance* programme.

   Transparency in the application of different methodologies is essential to ensure consistency in decision-making, ease of understanding, fairness and rationality. The assumptions made, the uncertainties, and the effect of these on the interpretation of the results, should be documented.

   The design of the *surveillance* programme will depend on the epidemiological circumstances and it should be planned and implemented in accordance with this chapter and Article 1.4.6.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.

   The *surveillance* programme should demonstrate absence of *infection* with high pathogenicity avian influenza viruses during the preceding 12 months in susceptible *poultry* populations (vaccinated and non-vaccinated).

   The design of the sampling strategy should include an epidemiologically appropriate design prevalence. The design prevalence and desired level of confidence in the results will determine the sample size. The Member Country should justify the choice of design prevalence and confidence level used on the basis of the stated objectives of the *surveillance* and the epidemiological situation.

   The sampling strategy may be risk-based if scientific evidence is available, and provided, for the quantification of risk factors. Specific risks could include those 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, the presence of more than one species at the *establishment* and poor *biosecurity* in place.

Data from different *surveillance* activities can be included to increase the sensitivity of the *surveillance* system. If this is to be done, data from structured (e.g. surveys and active *surveillance*) and non-structured (e.g. passive *surveillance*) sources should be combined and the sensitivity of each activity should be quantified in order to be able to quantify the sensitivity of the overall *surveillance* system.

The *surveillance* programme should include *surveillance* for high pathogenicity avian influenza viruses in birds other than *poultry*, including *wild* birds, and *monitoring* of low pathogenicity avian influenza viruses in *poultry*, in order to ensure that *biosecurity* and control measures are fit for purpose.

Documentation of 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 practise 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. Based on the epidemiology of avian influenza in the country, *zone* or *compartment*, a decision may be reached to vaccinate only certain species or other *poultry subpopulations*.

In all vaccinated *flocks* tests should be performed to ensure the absence of virus circulation. The tests should be repeated at a frequency that is proportionate to the *risk* in the country, *zone* or *compartment*. The use of sentinel *poultry* may provide further confidence in the absence of virus circulation.

Member Countries seeking the demonstration of freedom from high pathogenicity avian influenza in vaccinated population should refer to the chapter on avian influenza (*infection* with avian influenza viruses) in the *Terrestrial Manual*.

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

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 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. The use of sentinel birds may facilitate the interpretation of *surveillance* results.

Populations under this *surveillance* programme should include:

a) *establishments* in the proximity of the *outbreaks*;

b) *establishments* epidemiologically linked to the *outbreaks*;

c) *poultry* used to re-populate affected *establishments*;

d) any *establishments* where preventive depopulation has been carried out.

Article 10.4.29.

Surveillance of wild bird populations

Passive *surveillance*, i.e. sampling of birds found dead, is an appropriate method of *surveillance* in *wild* birds because *infection* with high pathogenicity avian influenza can be associated with mortality in some species. Mortality events, or clusters of birds found dead should be reported to the *Veterinary Services* and investigated, including through the collection and submission of samples to a *laboratory* for appropriate tests.

Active *surveillance*, i.e. sampling of live *wild* birds, may be necessary for detection of some strains of high pathogenicity avian influenza viruses that produce *infection* without mortality in *wild* birds. Furthermore, it increases knowledge of the ecology and evolution of avian influenza viruses.

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

Surveillance in *wild* birds should be enhanced by raising awareness, and by 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 10.4.30.

Monitoring of low pathogenicity avian influenza in poultry populations

Outbreaks of low pathogenicity avian influenza viruses can be managed at the *establishment* level; however, spread to other *poultry establishments* increases the risk of virus mutation, particularly if it is not detected and managed. Therefore, a *monitoring* system should be in place.

Monitoring the presence and types of low pathogenicity avian influenza viruses can be achieved through a combination of clinical investigation when *infection* is suspected because of changes in production parameters, such as reductions in egg production or *feed* and water intake, and active serological and virological *surveillance*, which can be supported by the information obtained by the *surveillance* system for high pathogenicity avian influenza.

Serological and virological *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 whether there is clustering of infected *flocks* regardless of whether the seropositive birds are still present at the *establishment* or whether active virus *infection* has been detected. Hence, *monitoring* of low pathogenicity avian influenza will also enhance early detection of high pathogenicity avian influenza.

NB: FIRST ADOPTED IN 1998; MOST RECENT UPDATE ADOPTED IN 2024.

GLOSSARY

For the purposes of the *Terrestrial Code*:

ANIMAL

means a mammal, reptile, bird or bee.

ANIMAL FOR BREEDING OR REARING

means a domesticated or confined *animal* which is not intended for *slaughter* within a short time.

ANIMAL FOR SLAUGHTER

means an *animal* intended for *slaughter* within a short time, under the control of the relevant *Competent Authority*.

ANIMAL HANDLER

means a person with a knowledge of the behaviour and needs of *animals* who, with appropriate experience and a professional and positive response to an *animal*'s needs, can achieve effective management and good *welfare*. Competence should be gained through formal training or practical experience.

ANIMAL HEALTH MANAGEMENT

means a system designed to optimise the physical and behavioural health and welfare of *animals*. It includes the prevention, treatment and control of diseases and conditions affecting the individual *animal* and *herd* or *flock*, including the recording of illness, injuries, mortalities and medical treatments where appropriate.

ANIMAL HEALTH STATUS

means the status of a country, *zone* or *compartment* with respect to an animal disease in accordance with the criteria listed in the relevant disease-specific chapter or Chapter 1.4. of the *Terrestrial Code*.

ANIMAL IDENTIFICATION

means the combination of the identification and *registration* of an *animal* individually, with a unique identifier, or collectively by its *epidemiological unit* or group, with a unique group identifier.

ANIMAL IDENTIFICATION SYSTEM

means the inclusion and linking of components such as identification of *establishments* or owners, the persons responsible for the *animals*, movements and other records with *animal identification*.

ANIMAL PRODUCT

means any part of an *animal*, or a raw or manufactured product containing any material derived from *animals*, excluding *germinal products*, *biological products* and *pathological material*.

ANIMAL TRACEABILITY

means the ability to follow an *animal* or group of *animals* during all stages of its life.

ANIMAL WELFARE

means the physical and mental state of an *animal* in relation to the conditions in which it lives and dies.

ANTIMICROBIAL AGENT

means a naturally occurring, semi-synthetic or synthetic substance that exhibits antimicrobial activity (kill or inhibit the growth of micro-organisms) at concentrations attainable *in vivo*. Anthelmintics and substances classed as disinfectants or antiseptics are excluded from this definition.

APIARY

means a *beehive* or group of *beehives* whose management allows them to be considered as a single *epidemiological unit*.

APPROVED

means officially approved, accredited or registered by the *Veterinary Authority*.

BEEHIVE

means a structure for the keeping of honey bee colonies that is being used for that purpose, including frameless hives, fixed frame hives and all designs of moveable frame hives (including nucleus hives), but not including packages or cages used to confine bees for the purposes of transport or isolation.

BIOLOGICAL PRODUCT

means a product of animal or microorganism origin, used in the diagnosis of diseases, for treatment, control and prevention of diseases, or in the collection and processing of *germinal products*.

BIOSECURITY

means a set of management and physical measures designed to reduce the *risk* of introduction, establishment and spread of animal diseases, *infections* or *infestations* to, from and within an animal population.

BIOSECURITY PLAN

means a plan that identifies potential pathways for the introduction and spread of disease in a *zone* or *compartment*, and describes the measures which are being or will be applied to mitigate the disease *risks*, if applicable, in accordance with the recommendations in the *Terrestrial Code*.

BORDER POST

means any airport, or any port, railway station or road check-point open to *international trade* of *commodities*, where import veterinary inspections can be performed.

CAPTIVE WILD [ANIMAL]

means an *animal* that has a phenotype not significantly affected by human selection but that is captive or otherwise lives under or requires human supervision or control.

CASE

means an individual *animal* infected by a pathogenic agent, with or without clinical signs.

CASINGS

means intestines and bladders that, after cleaning, have been processed by tissue scraping, defatting and washing, and have been treated with salt.

COLLECTION CENTRE

means a facility approved by the *Veterinary Authority* for the collection of oocytes or embryos and used exclusively for donor animals which meet the conditions of the *Terrestrial Code*.

COMMODITY

means a live *animal*, an *animal product*, *germinal products*, a *biological product* or *pathological material*.

COMPARTMENT

means an animal *subpopulation* contained in one or more *establishments*, separated from other susceptible *populations* by a common *biosecurity* management system, and with a specific *animal health status* with respect to one or more *infections* or *infestations* for which the necessary *surveillance*, *biosecurity* and control measures have been applied for the purposes of *international trade* or disease prevention and control in a country or *zone*.

COMPETENT AUTHORITY

means a Governmental Authority of a Member Country having the responsibility in the whole or part of the territory for the implementation of certain standards of the *Terrestrial Code*.

CONTAINER

means a non-self-propelled receptacle or other rigid structure for holding *animals* during a *journey* by one or several means of transport.

CONTAINMENT ZONE

means an *infected zone* defined within a previously free country or *zone*, which includes all suspected or confirmed *cases* that are epidemiologically linked and where movement control, *biosecurity* and *sanitary measures* are applied to prevent the spread of, and to eradicate, the *infection* or *infestation*.

DAY-OLD BIRDS

means birds aged not more than 72 hours after hatching.

DISINFECTION

means the application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; this applies to premises, *vehicles* and different objects which may have been directly or indirectly contaminated.

DISINFESTATION

means the application of procedures intended to eliminate *infestation*.

DISTRESS

means the state of an animal, that has been unable to adapt to stressors, and that manifests as abnormal physiological or behavioural responses. It can be acute or chronic and may result in pathological conditions.

EARLY WARNING SYSTEM

means a system for the timely detection, reporting and communication of occurrence, incursion or emergence of diseases, *infections* or *infestations* in a country, *zone* or *compartment*.

EMERGING DISEASE

means a new occurrence in an *animal* of a disease, *infection* or *infestation*, causing a significant impact on animal or public health resulting from:

a) a change of a known pathogenic agent or its spread to a new geographic area or species; or

b) a previously unrecognised pathogenic agent or disease diagnosed for the first time.

EPIDEMIOLOGICAL UNIT

means a group of *animals* with the same likelihood of exposure to a pathogenic agent. In certain circumstances, the epidemiological unit may be a single *animal*.

ERADICATION

means the elimination of a pathogenic agent from a country or *zone*.

ESTABLISHMENT

means the premises in which *animals* are kept.

EUTHANASIA

means the *killing* of an *animal* using a method that causes a rapid and irreversible loss of consciousness with minimum *pain* and *distress*.

EXPORTING COUNTRY

means a country from which *commodities* are sent to another country.

FEED

means any material (single or multiple), whether processed, semi-processed or raw, which is intended to be fed directly to terrestrial *animals* (except bees).

FEED INGREDIENT

means a component part or constituent of any combination or mixture making up a *feed*, whether or not it has a nutritional value in the *animal*'s diet, including feed additives. Ingredients are of plant (including aquatic plants) or terrestrial or aquatic animal origin, or other organic or inorganic substances.

FERAL [ANIMAL]

means an *animal* of a domesticated species that lives without requiring human supervision or control.

FLOCK

means a number of *animals* of one kind kept together under human control or a congregation of gregarious *wild animals*. A *flock* is usually regarded as an *epidemiological unit*.

FREE COMPARTMENT

means a *compartment* in which the absence of the animal pathogenic agent causing the disease under consideration has been demonstrated by all requirements specified in the *Terrestrial Code* for free status being met.

FREE-ROAMING DOG

means any *owned dog* or unowned dog that is without direct human supervision or control, including *feral* dogs.

FREE ZONE

means a *zone* in which the absence of a specific *infection* or *infestation* in an animal *population* has been demonstrated in accordance with the relevant requirements of the *Terrestrial Code*.

FRESH MEAT

means *meat* that has not been subjected to any treatment irreversibly modifying its organoleptic and physicochemical characteristics. This includes frozen *meat*, chilled *meat*, minced *meat* and mechanically recovered *meat*.

GERMINAL PRODUCTS

means animal semen, oocytes, embryos or *hatching eggs*.

GOOD MANUFACTURING PRACTICE

means a production and testing practice recognised by the *Competent Authority* to ensure the quality of a product.

HATCHING EGGS

means fertilised bird eggs, suitable for incubation and hatching.

HAZARD

means a biological, chemical or physical agent in, or a condition of, an *animal* or animal product with the potential to cause an adverse health effect.

HEADQUARTERS

means the Permanent Secretariat of the World Organisation for Animal Health located at:

12, rue de Prony, 75017 Paris, FRANCE
Telephone: 33-(0)1 44 15 18 88
Fax: 33-(0)1 42 67 09 87
Electronic mail: woah@woah.org
WWW: http://www.woah.org

HERD

means a number of *animals* of one kind kept together under human control or a congregation of gregarious *wild animals*. A *herd* is usually regarded as an *epidemiological unit*.

IMPORTING COUNTRY

means a country that is the final destination to which *commodities* are sent.

INCIDENCE

means the number of new *cases* or *outbreaks* of a disease that occur in a population at risk in a particular geographical area within a defined time interval.

INCUBATION PERIOD

means the longest period that elapses between the introduction of the pathogenic agent into the *animal* and the occurrence of the first clinical signs of the disease.

INFECTED ZONE

means a *zone* either in which an *infection* or *infestation* has been confirmed, or one that is defined as such in the relevant chapters of the *Terrestrial Code*.

INFECTION

means the entry and development or multiplication of a pathogenic agent in the body of humans or *animals*.

INFECTIVE PERIOD

means the longest period during which an affected *animal* can be a source of *infection*.

INFESTATION

means the external invasion or colonisation of *animals* or their immediate surroundings by arthropods, which may cause clinical signs or are potential *vectors* of pathogenic agents.

INTERNATIONAL TRADE

means importation, exportation and transit of *commodities*.

INTERNATIONAL VETERINARY CERTIFICATE

means a certificate, issued in accordance with Chapter 5.2., describing the animal health and public health requirements that are fulfilled by the exported *commodities*.

JOURNEY

An *animal* transport journey commences when the first *animal* is loaded onto a *vehicle/vessel* or into a *container* and ends when the last *animal* is unloaded, and includes any stationary resting/holding periods. The same *animals* do not commence a new journey until after a suitable period for rest and recuperation, with adequate *feed* and water.

KILLING

means any procedure that causes the death of an *animal*.

LABORATORY

means a properly equipped institution staffed by technically competent personnel under the control of a specialist in veterinary diagnostic methods, who is responsible for the validity of the results. The *Veterinary Authority* approves and monitors such laboratories with regard to the diagnostic tests required for *international trade*.

LAIRAGE

means pens, yards and other holding areas used for accommodating *animals* in order to give them necessary attention (such as water, *feed*, rest) before they are moved on or used for specific purposes including *slaughter*.

LISTED DISEASE

means a disease, *infection* or *infestation* listed in Chapter 1.3. after adoption by the World Assembly of Delegates.

LOADING/UNLOADING

Loading means the procedure of moving *animals* onto a *vehicle/vessel* or into a *container* for transport purposes, while unloading means the procedure of moving *animals* off a *vehicle/vessel* or out of a *container*.

MARKET

means a place where *animals* are assembled for the purposes of trade or sale.

MEAT

means all edible parts of an *animal*.

MEAT PRODUCTS

means *meat* that has been subjected to a treatment irreversibly modifying its organoleptic and physicochemical characteristics.

MILK

means the normal mammary secretion of milking *animals* obtained from one or more milkings without either addition to it or extraction from it.

MILK PRODUCT

means the product obtained by any processing of *milk*.

MONITORING

means the intermittent performance and analysis of routine measurements and observations, aimed at detecting changes in the environment or health status of a *population*.

NOTIFIABLE DISEASE

means a disease listed by the *Veterinary Authority*, and that, as soon as detected or suspected, should be brought to the attention of this *Authority*, in accordance with national regulations.

NOTIFICATION

means the procedure by which:

a) the *Veterinary Authority* informs the *Headquarters*,

b) the *Headquarters* inform the *Veterinary Authority*,

of the occurrence of disease, *infection* or *infestation* in accordance with Chapter 1.1.

OFFICIAL CONTROL PROGRAMME

means a programme which is approved, and managed or supervised by the *Veterinary Authority* of a Member Country for the purposes of controlling a *vector*, pathogenic agent or disease by specific measures applied throughout that Member Country, or within a *zone* or *compartment* of that Member Country.

OFFICIAL VETERINARIAN

means a *veterinarian* authorised by the *Veterinary Authority* of the country to perform certain designated official tasks associated with animal health or public health and inspections of *commodities* and, when appropriate, to certify in accordance with Chapters 5.1. and 5.2.

OFFICIAL VETERINARY CONTROL

means the operations whereby the *Veterinary Services*, knowing the location of the *animals* and after taking appropriate actions to identify their owner or responsible keeper, are able to apply appropriate animal health measures, as required. This does not exclude other responsibilities of the *Veterinary Services* e.g. food safety.

OUTBREAK

means the occurrence of one or more *cases* in an *epidemiological unit*.

OWNED DOG

means a dog for which a person claims responsibility.

PAIN

means an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It may elicit protective actions, result in learned avoidance and *distress* and may modify species-specific traits of behaviour, including social behaviour.

PATHOLOGICAL MATERIAL

means samples obtained from live or dead *animals*, containing or suspected of containing infectious or parasitic agents, to be sent to a *laboratory*.

PLACE OF SHIPMENT

means the place where the *commodities* are loaded into the *vehicle* or handed to the agency that will transport them to another country.

POPULATION

means a group of *units* sharing a common defined characteristic.

POULTRY

means all birds reared or kept in captivity for the production of any commercial animal products or for breeding for this purpose, fighting cocks used for any purpose, and all birds used for restocking supplies of game or for breeding for this purpose, until they are released from captivity.

Birds that are kept in a single household, the products of which are used within the same household exclusively, are not considered *poultry*, provided that they have no direct or indirect contact with *poultry* or *poultry* facilities.

Birds that are kept in captivity for other reasons, including those that are kept for shows, racing, exhibitions, zoological collections and competitions, and for breeding or selling for these purposes, as well as pet birds, are not considered *poultry*, provided that they have no direct or indirect contact with *poultry* or *poultry* facilities.

PRE-JOURNEY PERIOD

means the period during which *animals* are identified, and often assembled for the purposes of *loading* them.

PREVALENCE

means the total number of *cases* or *outbreaks* of a disease that are present in a population at risk, in a particular geographical area, at one specified time or during a given period.

PROTEIN MEAL

means any final or intermediate solid protein-containing product, obtained when animal tissues are rendered, excluding peptides of a molecular mass less than 10,000 daltons and amino-acids.

PROTECTION ZONE

means a *zone* where specific *biosecurity* and *sanitary measures* are implemented to prevent the entry of a pathogenic agent into a free country or *zone* from a neighbouring country or *zone* of a different *animal health status*.

QUALITATIVE RISK ASSESSMENT

means an assessment where the outputs on the likelihood of the outcome or the magnitude of the consequences are expressed in qualitative terms such as 'high', 'medium', 'low' or 'negligible'.

QUANTITATIVE RISK ASSESSMENT

means an assessment where the outputs of the *risk assessment* are expressed numerically.

QUARANTINE STATION

means an establishment under the control of the *Veterinary Authority* where *animals* are maintained in isolation with no direct or indirect contact with other *animals*, to ensure that there is no transmission of specified pathogenic agents outside the establishment while the *animals* are undergoing observation for a specified length of time and, if appropriate, testing or treatment.

REGISTRATION

is the action by which information on *animals* (such as identification, animal health, movement, certification, epidemiology, *establishments*) is collected, recorded, securely stored and made appropriately accessible and able to be utilised by the *Competent Authority*.

RESPONSIBLE DOG OWNERSHIP

means the situation whereby a person accepts and commits to perform various duties in accordance with the legislation in place and focused on the satisfaction of the behavioural, environmental and physical needs of a dog and to the prevention of risks (aggression, disease transmission or injuries) that the dog may pose to the community, other *animals* or the environment.

RESTING POINT

means a place where the *journey* is interrupted to rest, *feed* or water the *animals*; the *animals* may remain in the *vehicle*/*vessel* or *container*, or be unloaded for these purposes.

RESTRAINT

means the application to an *animal* of any procedure designed to restrict its movements.

RISK

means the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health.

RISK ANALYSIS

means the process composed of *hazard* identification, *risk assessment*, *risk management* and *risk communication*.

RISK ASSESSMENT

means the evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a *hazard*.

RISK COMMUNICATION

is the interactive transmission and exchange of information and opinions throughout the *risk analysis* process concerning *risk*, *risk*-related factors and *risk* perceptions among *risk* assessors, *risk* managers, *risk* communicators, the general public and other interested parties.

RISK MANAGEMENT

means the process of identifying, selecting and implementing measures that can be applied to reduce the level of *risk*.

SAFE COMMODITY

means a *commodity* that can be traded without the need for *risk* mitigation measures specifically directed against a particular *listed disease*, *infection* or *infestation* and regardless of the status of the country or *zone* of origin for that disease, *infection* or *infestation*.

SANITARY MEASURE

means a measure, such as those described in various chapters of the *Terrestrial Code*, designed to protect animal or human health or life within the whole territory or a *zone* of a Member Country from *risks* arising from the entry, establishment or spread of a *hazard*.

SEMEN COLLECTION CENTRE

means an *approved* facility that meets the conditions set out in the *Terrestrial Code* for the collection, processing and storage of semen.

SLAUGHTER

means the *killing* of an *animal* primarily intended for human consumption.

SLAUGHTERHOUSE/ABATTOIR

means premises, including facilities for moving or lairaging *animals*, used for the *slaughter* of *animals* to produce animal products and approved by the relevant *Competent Authority*.

SPACE ALLOWANCE

means the measure of the floor area and height allocated per individual or body weight of *animals*.

SPECIFIC SURVEILLANCE

means the *surveillance* targeted to a specific disease or *infection*.

STAMPING-OUT POLICY

means a policy designed to eliminate an *outbreak* by carrying out under the authority of the *Veterinary Authority* the following:

a) the *killing* of the *animals* which are affected and those suspected of being affected in the *herd* or *flock* and, where appropriate, those in other *herds* or *flocks* which have been exposed to *infection* by direct animal to animal contact, or by indirect contact with the causal pathogenic agent; *animals* should be killed in accordance with Chapter 7.6.;

b) the disposal of carcasses and, where relevant, animal products by rendering, burning or burial, or by any other method described in Chapter 4.13.;

c) the cleansing and *disinfection* of *establishments* through procedures defined in Chapter 4.14.

STOCKING DENSITY

means the number or body weight of *animals* per unit area on a *vehicle/vessel* or *container*.

STUNNING

means any procedure that causes loss of consciousness for the purpose of *killing* without avoidable *distress*, fear and *pain*.

SUBPOPULATION

means a distinct part of a *population* identifiable in accordance with specific common animal health characteristics.

SURVEILLANCE

means the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information so that action can be taken.

TERRESTRIAL CODE

means the WOAH *Terrestrial Animal Health Code*.

TERRESTRIAL MANUAL

means the WOAH *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.

TRANSIT COUNTRY

means a country through which *commodities* destined for an *importing country* are transported or in which a stopover is made at a *border post*.

UNIT

means an individually identifiable element used to describe, for example, the members of a *population* or the elements selected when sampling; examples of *units* include individual *animals*, *herds*, *flocks* and *apiaries*.

VACCINATION

means the administration of a vaccine, in accordance with the manufacturer's instructions and the *Terrestrial Manual*, when relevant, with the intention of inducing immunity in an *animal* or group of *animals* against one or more pathogenic agents.

VECTOR

means an insect or any living carrier that transports an infectious agent from an infected individual to a susceptible individual or its food or immediate surroundings. The organism may or may not pass through a development cycle within the *vector*.

VEHICLE/VESSEL

means any means of conveyance including train, truck, aircraft or ship that is used for carrying *animals*.

VETERINARIAN

means a person with appropriate education, registered or licensed by the relevant *veterinary statutory body* of a country to practice veterinary medicine/science in that country.

VETERINARY AUTHORITY

means the Governmental Authority of a Member Country having the primary responsibility in the whole territory for coordinating the implementation of the standards of the *Terrestrial Code*.

VETERINARY LEGISLATION

means laws, regulations and all associated legal instruments that pertain to the veterinary domain.

VETERINARY MEDICINAL PRODUCT

means any product with approved claims to having a prophylactic, therapeutic or diagnostic effect or to alter physiological functions when administered or applied to an *animal*.

VETERINARY PARAPROFESSIONAL

means a person who, for the purposes of the *Terrestrial Code*, is authorised by the *veterinary statutory body* to carry out certain designated tasks (dependent upon the category of *veterinary paraprofessional*) in a territory, and delegated to them under the responsibility and direction of a *veterinarian*. The tasks for each category of *veterinary paraprofessional* should be defined by the *veterinary statutory body* depending on qualifications and training, and in accordance with need.

VETERINARY SERVICES

means the combination of governmental and non-governmental individuals and organisations that perform activities to implement the standards of the *Terrestrial Code*.

VETERINARY STATUTORY BODY

means an autonomous regulatory body for *veterinarians* and *veterinary paraprofessionals*.

WILD [ANIMAL]

means an *animal* that has a phenotype unaffected by human selection and lives independently without requiring human supervision or control.

WILDLIFE

means *feral animals*, *captive wild animals* and *wild animals*.

ZONE

means a part of a country defined by the *Veterinary Authority*, containing an animal *population* or *subpopulation* with a specific *animal health status* with respect to an *infection* or *infestation* for the purposes of *international trade* or disease prevention or control.

NB: FIRST ADOPTED IN 1968; MOST RECENT UPDATE ADOPTED IN 2024.

SECTION 5.

TRADE MEASURES, IMPORT/EXPORT PROCEDURES AND VETERINARY CERTIFICATION

CHAPTER 5.1.

GENERAL OBLIGATIONS RELATED TO CERTIFICATION

Article 5.1.1.

Safety of *international trade* in *animals* and animal products depends on a combination of factors which should be taken into account to ensure unimpeded trade, without incurring unacceptable *risks* to human and animal health.

Because of differences between countries in their animal health situations, various options are offered by the *Terrestrial Code*. The animal health situation in the *exporting country*, in the *transit country* or *countries* and in the *importing country* should be considered before determining the requirements for trade. To maximise harmonisation of the sanitary aspects of *international trade*, *Veterinary Authorities* of Member Countries should base their import requirements on the standards of WOAH.

These requirements should be included in the model certificates approved by WOAH which are included from Chapters 5.10. to 5.12.

Certificates should be exact and concise, and should clearly convey the requirements of the *importing country*. For this purpose, prior consultation between *Veterinary Authorities* of *importing* and *exporting countries* may be necessary. It enables the setting out of the exact requirements so that the signing *veterinarian* can, if necessary, be given a note of guidance explaining the understanding between the *Veterinary Authorities* involved.

The certification requirements should not include conditions for diseases that are not transmitted by the *commodity* concerned. The certificate should be signed in accordance with Chapter 5.2.

When officials of a *Veterinary Authority* wish to visit another country for matters of professional interest to the *Veterinary Authority* of the other country, the latter should be informed.

Article 5.1.2.

Responsibilities of the importing country

1) The import requirements included in the *international veterinary certificate* should assure that *commodities* introduced into the *importing country* comply with the standards of WOAH. *Importing countries* should align their requirements with the recommendations in the relevant standards of WOAH. If there are no such recommendations or if the country chooses a level of protection requiring measures more stringent than the standards of WOAH, these should be based on an import *risk analysis* conducted in accordance with Chapter 2.1.

2) The *international veterinary certificate* should not include requirements for the exclusion of pathogenic agents or animal diseases which are present in the *importing country* and are not subject to any *official control programme*. The measures imposed on imports to manage the *risks* posed by a specific pathogenic agent or disease should not be more stringent than those applied as part of the *official control programme* operating within the *importing country*.

3) The *international veterinary certificate* should not include measures against pathogenic agents or diseases which are not WOAH listed, unless the *importing country* has demonstrated through import *risk analysis*, carried out in accordance with Section 2., that the pathogenic agent or disease poses a significant *risk* to the *importing country*.

4) The transmission by the *Veterinary Authority* of certificates or the communication of import requirements to persons other than the *Veterinary Authority* of another country, necessitates that copies of these documents are also sent to the *Veterinary Authority*. This important procedure avoids delays and difficulties which may arise between traders and *Veterinary Authorities* when the authenticity of the certificates or permits is not established.

   This procedure is under the responsibility of *Veterinary Authorities*. However, it can be undertaken by private sector *veterinarians* at the place of origin of the *commodities* when this practice is the subject of appropriate approval and authentication by the *Veterinary Authority*.

5) Situations may arise which result in changes to the consignee, identification of the means of transportation, or *border post* after a certificate is issued. Because these do not change the animal or public health status of the consignment, they should not prevent the acceptance of the certificate.

Article 5.1.3.

Responsibilities of the exporting country

1) An *exporting country* should, on request, supply the following to *importing countries*:
   a) information on the animal health situation and national animal health information systems to determine whether that country is free or has *zones* or *compartments* free from *listed diseases*, including the regulations and procedures in force to maintain its free status;
   b) regular and prompt information on the occurrence of *notifiable diseases*;
   c) details of the country's ability to apply measures to control and prevent the relevant *listed diseases*;
   d) information on the structure of the *Veterinary Services* and the authority which they exercise in accordance with Chapters 3.2. and 3.3.;
   e) technical information, particularly on biological tests and vaccines applied in all or part of the national territory.
2) *Veterinary Authorities* of *exporting countries* should:
   a) have official procedures for authorisation of certifying *veterinarians*, defining their functions and duties as well as conditions of oversight and accountability, including possible suspension and termination of the authorisation;
   b) ensure that the relevant instructions and training are provided to certifying *veterinarians*;
   c) monitor the activities of the certifying *veterinarians* to verify their integrity and impartiality.
3) The *Veterinary Authority* of the *exporting country* is ultimately accountable for veterinary certification used in *international trade*.

Article 5.1.4.

Responsibilities in case of an incident related to importation

1) *International trade* involves a continuing ethical responsibility. Therefore, if within the recognised *incubation periods* of the various diseases subsequent to an export taking place, the *Veterinary Authority* becomes aware of the appearance or reappearance of a disease which has been specifically included in the *international veterinary certificate*, there is an obligation for this *Authority* to notify the *importing country*, so that the imported *commodities* may be inspected or tested and appropriate action be taken to limit the spread of the disease should it have been inadvertently introduced.

2) If a disease condition appears in imported *commodities* within a time period after importation consistent with the recognised *incubation period* of the disease, the *Veterinary Authority* of the *exporting country* should be informed so as to enable an investigation to be made, since this may be the first available information on the occurrence of the disease in a previously free *herd* or *flock*. The *Veterinary Authority* of the *importing country* should be informed of the result of the investigation since the source of *infection* may not be in the *exporting country*.

3) In case of suspicion, on reasonable grounds, that an official certificate may be fraudulent, the *Veterinary Authorities* of the *importing country* and *exporting country* should conduct an investigation. Consideration should also be given to notifying any third country that may have been implicated. All associated consignments should be kept under official control, pending the outcome of the investigation. The *Veterinary Authorities* of all countries involved should fully cooperate with the investigation. If the certificate is found to be fraudulent, every effort should be made to identify those responsible so that appropriate action can be taken in accordance with the relevant legislation.

NB: FIRST ADOPTED IN 1982; MOST RECENT UPDATE ADOPTED IN 2024.

CHAPTER 3.3.4.

AVIAN INFLUENZA (INCLUDING INFECTION WITH HIGH PATHOGENICITY AVIAN INFLUENZA VIRUSES)

SUMMARY

Influenza A is caused by specified viruses that are members of the family Orthomyxoviridae *and placed in the genus* Alphainfluenzavirus (Influenzavirus *A or influenza A virus). There are seven influenza genera but only influenza A viruses are known to infect birds. Diagnosis is by isolation of the virus or by detection and characterisation of fragments of its genome. This is because infections in birds can give rise to a wide variety of clinical signs that may vary according to the host, strain of virus, the host's immune status, presence of any secondary exacerbating organisms and environmental conditions.*

Detection of the agent: *Suspensions in antibiotic solution of oropharyngeal and cloacal swabs (or faeces) taken from live birds, or of faeces and pooled samples of organs from dead birds, are inoculated into the allantoic cavity of 9- to 11-day-old embryonated chicken eggs. The eggs are incubated at 37°C (range 35–39°C) for 2–7 days. The allantoic fluid of any eggs containing dead or dying embryos during the incubation and all eggs at the end of the incubation period are tested for the presence of haemagglutinating activity. The presence of influenza A virus can be confirmed by an immunodiffusion test between concentrated virus and an antiserum to the nucleoprotein and/or matrix antigens, both of which are common to all influenza A viruses, or by real-time reverse-transcription polymerase chain reaction (real-time RT-PCR) on the allantoic fluids. Isolation in embryos has largely been replaced for initial diagnosis by direct detection in samples, of one or more segments of the influenza A genome using real-time RT-PCR or other validated molecular techniques.*

For serological subtyping of the virus, a reference laboratory should conduct haemagglutination and neuraminidase inhibition tests against a battery of polyclonal or monospecific antisera to each of the 16 haemagglutinin (H1–16) and 9 neuraminidase (N1–9) subtypes of influenza A virus. Alternatively, the genome of specific H and N subtypes is identified using RNA detection technologies with subtype specific primers and probes (e.g. real-time RT-PCR) or sequencing and phylogenetic analysis.

As the general term 'highly pathogenic avian influenza' and the historical term 'fowl plague' refer to infection with high pathogenicity strains of influenza A virus, it is necessary to assess the pathogenicity of Influenza A virus isolates for domestic poultry. All naturally occurring high pathogenicity avian influenza (HPAI) strains isolated to date have been either of the H5 or H7 subtype, with a subset of H5 or H7 isolates being of low pathogenicity. The methods used for the determination of strain virulence for birds have evolved over recent years with a greater understanding of the molecular basis of pathogenicity. Regardless of their pathogenicity for chickens, H5 or H7 viruses with a HA0 cleavage site amino acid sequence similar to any of those that have been observed in high pathogenicity viruses are considered to be influenza A viruses with high pathogenicity. H5 and H7 isolates that are not highly pathogenic for chickens and do not have an HA0 cleavage site amino acid sequence similar to any of those that have been observed in highly pathogenic viruses are considered to have low pathogenicity. However in some circumstances it is necessary to verify high or low pathogenicity of a virus isolate using the intravenous inoculation of a minimum of eight susceptible 4- to 8-week-old chickens with infectious virus; strains are considered to be of high pathogenicity if they cause more than 75% mortality within 10 days, or inoculation of 10 susceptible 4- to 8-week-old chickens resulting in an intravenous pathogenicity index (IVPI) of greater than 1.2. Characterisation of suspected highly pathogenic strains of the virus should be conducted in a virus-secure biocontainment laboratory. Low pathogenicity avian influenza (LPAI) in poultry may be accompanied

by a sudden and unexpected increase in virulence (emerging disease) or have proven natural transmission to humans associated with severe consequences. In these disease scenarios there should be formal monitoring in relevant poultry populations by national authorities. The occurrence of H5 and H7 low pathogenicity avian influenza viruses should be monitored as some have the potential to mutate into high pathogenicity avian influenza viruses.

Serological tests: *As all influenza A viruses have antigenically similar nucleoprotein and matrix antigens, these are preferred targets of influenza A group serological methods. Enzyme-linked immunosorbent assays (ELISA) are widely used to detect antibodies to these antigens in either host species-dependent (indirect) or species-independent (competitive) test formats. Haemagglutination inhibition tests have also been employed in routine diagnostic serology, but it is possible that this technique may miss some particular infections because the haemagglutinin is subtype specific.*

Requirements for vaccines: *The first use of vaccination in an avian influenza eradication programme was against LPAI. The programmes used inactivated oil-emulsion vaccines with the same haemagglutinin and neuraminidase subtypes as the circulating field virus, and infected flocks were identified by detection of virus or antibodies against the virus in non-vaccinated sentinel birds. During the 1990s the prophylactic use of inactivated oil-emulsion vaccines was employed in Mexico and Pakistan to control widespread outbreaks of HPAI and H5/H7 LPAI. During the 1999–2001 outbreak of H7 LPAI in Italy, an inactivated vaccine was used with the same (i.e. homologous) haemagglutinin subtype to the field virus, but with a different (i.e. heterologous) neuraminidase. This allowed the serological differentiation of non-infected vaccinated birds from vaccinated birds infected with the field virus and ultimately resulted in eradication of the field virus. Prophylactic use of H5 and H7 vaccines has been practised in parts of Italy, aimed at preventing H5/H7 LPAI infections, and several countries in Asia, Africa and the Middle East as an aid in controlling HPAI, in China (People's Rep. of) for H7N9, and in Mexico for H7N3 HPAI virus infections. HPAI viruses should not be used as the seed virus for production of vaccine.*

If LPAI and HPAI viruses are used in challenge studies, an appropriate level of containment should be used as determined by risk assessment.

A. INTRODUCTION

Influenza in birds is caused by infection with viruses of the family *Orthomyxoviridae* placed in the genus *Alphainfluenzavirus* (*influenzavirus A* or influenza A virus) (International Committee on Taxonomy of Viruses (ICTV), 2019). Influenza A viruses are the only orthomyxoviruses known to naturally affect birds (Swayne & Sims, 2020). Many species of birds have been shown to be susceptible to infection with influenza A viruses; aquatic birds form a major reservoir of these viruses, and the overwhelming majority of isolates have been of low pathogenicity (low virulence) for chickens and turkeys. Influenza A viruses have antigenically related nucleoprotein and matrix proteins, but are classified into subtypes on the basis of their haemagglutinin (H) and neuraminidase (N) antigens (World Health Organization Expert Committee, 1980). At present, 16 H subtypes (H1–H16) and 9 N subtypes (N1–N9) are recognised with proposed new subtypes (H17, H18) for influenza A viruses from bats in Guatemala (ICTV 2019; Swayne *et al.*, 2020; Tong *et al.*, 2013). To date, naturally occurring high pathogenicity influenza A viruses that produce acute clinical disease in chickens, turkeys and other birds of economic importance have been associated only with the H5 and H7 subtypes. Low pathogenicity H5 and H7 occur widely in poultry and aquatic wild birds, although intercontinental spread of HPAI has received greater attention in recent years. There is the risk of a H5 or H7 virus of low pathogenicity (H5/H7 low pathogenicity avian influenza [LPAI]) becoming highly pathogenic by mutation. Some avian influenza virus strains have caused sporadic zoonotic infections principally of H5, H7 and H9 subtypes and these three subtypes have been highlighted as potential pandemic risks should additional mutations occur that support sustained human-to-human transmission (Cox *et al.*, 2017).

Throughout this chapter of the *Terrestrial Manual*, the following terms will be used: 1) HPAI as an infection by an avian influenza virus that meets the definition of high pathogenicity, 2) LPAI as an infection with any H1–H16 avian influenza virus that is not of high pathogenicity, and 3) influenza A as an infection with any HPAI or LPAI virus.

Depending on the species, age and type of bird, specific characteristics of the viral strain involved, and on environmental factors, the highly pathogenic disease, in fully susceptible birds, may vary from one of sudden death with no overt clinical signs, to a more characteristic disease with variable clinical presentations including respiratory signs, such as ocular and nasal discharges, coughing, snicking and dyspnoea, swelling of the sinuses and/or head,

apathy, reduced vocalisation, marked reduction in feed and water intake, cyanosis of the unfeathered skin, wattles and comb, incoordination and nervous signs and diarrhoea (Swayne *et al.*, 2020). In laying birds, additional clinical features include a marked drop in egg production, usually accompanied by an increase in numbers of poor quality eggs. Typically, high morbidity is accompanied by high and rapidly escalating unexplained mortality. However, none of these signs can be considered pathognomonic. In certain host species such as Pekin ducks (*Anas platyrhynchos domesticus*) some HPAI viruses do not necessarily produce significant clinical disease. In addition, LPAI viruses which normally cause only a mild or no clinical disease, may in certain circumstances produce a spectrum of clinical signs, the severity of which may approach that of HPAI, particularly if exacerbating infections and/or adverse environmental conditions are present. Confirmatory diagnosis of the disease, therefore, depends on the isolation or detection of the causal virus and the demonstration that it fulfils one of the defined criteria described in Section B.1.1.1. Testing sera from suspect birds using antibody detection methods may supplement diagnosis, but these methods are not suitable for a definitive identification. Diagnosis for official control purposes is established on the basis of agreed official criteria for pathogenicity according to *in-vivo* tests or to molecular determinants (i.e. the presence of a cleavage site of the haemagglutinin precursor protein HA0 consistent with HPAI virus) and haemagglutinin subtyping. These definitions evolve as scientific knowledge of the disease increases.

HPAI should be subject to official control by national authorities. In addition LPAI, particularly H5 and H7 subtypes, may be subject to national or state/provincial control. The viruses that cause influenza A have the potential to spread from the laboratory if adequate levels of biosecurity and biosafety are not in place. Avian influenza viruses should be handled with appropriate measures as described in Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*. Biocontainment measures should be determined by risk analysis as described in Chapter 1.1.4. The measures required may vary among the subtypes and pathotypes of influenza A viruses, with higher level containment being indicated for some LPAI and HPAI viruses, and may require additional procedural, equipment and facility enhancements under specific conditions such as high virus concentrations, housing infected animals or conducting procedures with aerosol generating activities. Countries lacking access to such a specialised national or regional laboratory should send specimens to a WOAH Reference Laboratory.

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of avian influenza and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
	Detection of the agent[a]					
Virus isolation	+	+++	+	+++	+	–
Antigen detection	+	+	+	+	+	–
Real-time RT-PCR	++	+++	++	+++	++	–

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
	Detection of immune response					
AGID	+ (Influenza A)	+ (Influenza A)	++ (Influenza A)	+ (convalescent)	++ (Influenza A)	++ (Influenza A)
HI	+++ (H5 or H7)	++ (H5 or H7)	+++ (H5 or H7)	++ (convalescent)	+++ (H5 or H7)	+++ (H5 or H7)
ELISA	+	+	++	+ (convalescent)	++	++

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
RT-PCR = reverse-transcription polymerase chain reaction; AGID = agar gel immunodiffusion;
HI = haemagglutination inhibition test; ELISA = enzyme-linked immunosorbent assay.
[a]A combination of agent identification methods applied on the same clinical sample is recommended.

1. Detection of the agent

Identification of influenza A viruses as the cause of infections and disease in poultry and other birds requires a thorough diagnostic investigation to differentiate from similar diseases caused by other viral agents especially avian paramyxovirus type 1 (APMV-1). Individual influenza A and APMV-1 virus isolates vary greatly in virulence, causing various syndromes evident as subclinical infections, drops in egg production, respiratory disease, and severe and high mortality disease. The latter clinical syndrome can be caused by either HPAI or Newcastle disease viruses. Therefore, it is judicious to have a single sampling procedure and simultaneously conduct specific differentiating diagnostic tests for both influenza A and APMV-1 viruses on field samples to obtain an accurate aetiological diagnosis of a single agent or, on occasion, confirmation of dual infection.

1.1. Samples for virus isolation

Virus isolation is the reference method but is laborious and time intensive, used primarily for diagnosis of a first clinical case in an outbreak and to obtain virus isolates for further laboratory analysis.

For investigations of severe disease and high mortality in poultry flocks, it is usual to attempt virus isolation from recently dead birds or moribund birds that have been killed humanely. Samples taken from dead birds should include intestinal contents (faeces) or cloacal swabs and oropharyngeal or tracheal swabs. Samples from trachea, lungs, air sacs, intestine, spleen, caecal tonsils, kidney, brain, liver and heart should also be collected and processed either separately or as a pool. When pooling samples the brain should be collected and processed first (to avoid cross contamination with other tissue types) and kept separate as presence of virus in the brain may be an indicator of HPAI or NDV. Further pools should be made consistent with known virus tropisms between HPAI and LPAI, i.e. grouped at the level of respiratory, systemic and gastrointestinal.

Samples from live birds should include both oropharyngeal or tracheal and cloacal swabs, the latter should be visibly coated with faecal material. To avoid harming them, swabbing of small delicate birds should be done with the use of especially small swabs that are usually commercially available and intended for use in human paediatrics or the collection of fresh faeces may serve as an adequate alternative (caution that some influenza A viruses and type 1 avian paramyxoviruses in birds can have a strong respiratory tropism). Similar swab samples can be pooled from the same anatomical site (i.e. cloacal swabs with cloacal swabs, oropharyngeal swabs with oropharyngeal swabs), and most commonly pooling of 5 or occasionally more, if appropriately validated not to reduce sensitivity of detection, but specific swab types should be used (Spackman *et al.*, 2013). Further the type of swabs used may affect test sensitivity or validity with thin wire or plastic shafted swabs preferred.

The samples should be placed in isotonic phosphate-buffered saline (PBS), pH 7.0–7.4 with antibiotics or a solution containing protein and antibiotics. The antibiotics can be varied according to local conditions, but could be, for example, penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50 µg/ml) and mycostatin (1000 units/ml) for tissues and oropharyngeal or tracheal swabs, but at five-fold higher concentrations for faeces and cloacal swabs. It is important to re-adjust the pH of the solution to pH 7.0–7.4 following the addition of the antibiotics. It is recommended that a solution for transport of the swabs should contain protein to stabilise the virus (e.g. brain–heart infusion, up to 5% [v/v] cattle serum, 0.5% [w/v] bovine albumen or similar commercially available transport media). If control of *Chlamydophila* is desired, 0.05–0.1 mg/ml oxytetracycline should be included. Faeces and finely minced tissues should be prepared as 10–20% (w/v) suspensions in the antibiotic solution. Suspensions should be processed as soon as possible after incubation for 1–2 hours at room temperature. When immediate processing is impractical, samples may be stored at 4°C for up to 4 days. For prolonged storage, diagnostic samples and isolates should be kept at –80°C but for transport on dry ice (≤–50°C) is widely used. Repeated freezing and thawing should be avoided.

1.2. Virus isolation

The preferred method of growing influenza A viruses is by the inoculation of specific pathogen free (SPF) embryonated chicken eggs, or specific antibody negative (SAN) eggs. The supernatant fluids of faeces, swabs or tissue suspensions obtained through clarification by centrifugation at 1000 ***g*** for about 10 minutes at a temperature not exceeding 25°C. Clarified preparations can be inoculated using a number of routes including the amniotic sac, chorioallantoic sac or membrane (one of which is recommended for primary isolation) and in all cases allantoic sacs of three to five embryonated SPF or SAN chicken eggs of 9–11 days' incubation. The eggs are incubated at 37°C (range 35–39°C) for 2–7 days. Eggs containing dead or dying embryos as they arise, and all eggs remaining at the end of the incubation period, should first be chilled to 4°C for 4 hours or overnight. After checking that the embryos have died, the amnio-allantoic fluids should be recovered and tested with a screening test (such as haemagglutination [HA] test), influenza A type-specific test (such as agar gel immunodiffusion test [AGID] or solid-phase antigen-capture enzyme-linked immunosorbent assays [ELISA]) or influenza A subtype-specific test (such as haemagglutination inhibition [HI] and neuraminidase [N] inhibition [NI] tests) or a molecular test to detect influenza A specific nucleic acid signatures (such as real-time reverse transcription polymerase chain reaction [RT-PCR]) as described later (see Section B.1.2.2). Detection of HA activity, in bacteria-free amnio-allantoic fluids verified by microbiological assay, indicates a high probability of the presence of an influenza A virus or of an avian orthoavulavirus (formerly avian paramyxovirus). Fluids that give a negative reaction should be passaged into at least one further batch of eggs, and up to three passages.

Routine checks for bacterial contamination should be conducted by streaking samples in Luria Broth agar plates and reading these at 24 and 48 hours of incubation against a light source. BHI agar and blood agar plates may also be used. For larger numbers of sample initial culture could be in tryptose phosphate broth. Contaminated samples can be treated by incubation with increased antibiotic concentrations for 2–4 hours (gentamicin, penicillin g, and amphotericin b solutions at final concentrations to a maximum of 1 mg/ml, 10,000 U/ml, and 20 µg/ml, respectively). Samples heavily contaminated by bacteria that cannot be removed by centrifugation or controlled by antibiotics can be filtrated through 0.45 and 0.2 micron sterile filters. Filtration should be used only when other methods fail because aggregation may significantly reduce virus titre.

1.3. Virus identification

The presence of influenza A virus can be confirmed in AGID tests by demonstrating the presence of the nucleoprotein or matrix antigens, both of which are common to all influenza A viruses (see Section B.2.2). The antigens may be prepared by concentrating the virus from infective allantoic fluid or extracting the infected chorioallantoic membranes; these are tested against known positive antisera. Virus may be concentrated from infective allantoic fluid by ultracentrifugation, or by precipitation under acid conditions. The latter method consists of the addition of 1.0 M HCl to infective allantoic fluid until it is approximately pH 4.0. The mixture is placed in an ice bath for 1 hour and then clarified by centrifugation at 1000 ***g*** at 4°C. The supernatant fluid is discarded. The virus concentrates are resuspended in glycin/sarcosyl buffer: this consists of 1% (w/v) sodium lauroyl sarcosinate buffered to pH 9.0 with 0.5 M glycine. These concentrates contain both nucleoprotein and matrix polypeptides.

Preparations of nucleoprotein-rich antigen can also be obtained from chorioallantoic membranes for use in the AGID test (Beard, 1970). This method involves removal of the chorioallantoic membranes from infected eggs that have allantoic fluids with HA activity. The membranes are then homogenised or ground to a paste. This is subjected to three freeze–thaw cycles, followed by centrifugation at 1000 ***g*** for 10 minutes. The pellet is discarded and the supernatant is used as an antigen following treatment with 0.1% formalin or 1% betapropiolactone.

Use of the AGID test to demonstrate nucleoprotein or matrix antigens is a satisfactory way to indicate the presence of influenza A virus in amnioallantoic fluid, but lacks sensitivity compared to other methods including molecular (see Section 1.2.2) but various experimental and commercial rapid, solid-phase antigen-capture ELISAs (AC-ELISAs) are an effective alternative (Swayne et *al.*, 2020). Most AC-ELISAs have been approved and marketed to detect human influenza A virus in clinical specimens. Some have demonstrated effectiveness for detection of influenza A, but many of these commercial tests have had low sensitivity (Slomka et *al.*, 2012). Those validated for veterinary use are preferred.

Any HA activity of sterile fluids harvested from the inoculated eggs is most likely to be caused by an influenza A virus or an avian paramyxovirus, but a few strains of avian reovirus, as well as nonsterile fluid containing HA of bacterial origin can cause the agglutination of RBCs. There are currently 21 recognised serotypes of avian paramyxoviruses (ICTV, 2019). Most laboratories will have antiserum specific to Newcastle disease virus (avian paramyxovirus type 1, APMV1), and in view of its widespread occurrence and almost universal use as a live vaccine in poultry, it is best to evaluate its presence by haemagglutination inhibition (HI) tests (see Chapter 3.3.10 *Newcastle disease*).

Alternatively, the presence of influenza virus can be confirmed by the use of conventional RT-PCR or real-time RT-PCR using nucleoprotein-specific or matrix-specific conserved primers (Nagy et *al.*, 2020; Spackman *et al.*, 2002). Also, the presence of subtype H5 or H7 influenza virus can be confirmed by using H5- or H7-specific primers (Slomka et *al.*, 2007; Spackman et *al.*, 2002).

Antigenic subtyping can be accomplished by monospecific antisera prepared against purified or recombinant H and N subtype-specific proteins, used in HI and NI tests, or polyclonal antisera raised against a range of intact influenza viruses and used in HI and NI tests. For laboratories conducting the HI test to H subtype it is strongly recommended that two sera for each H subtype is used but with a heterologous N and should ideally use antisera to contemporary viruses relevant to the region in which the virus is detected. Subtyping can also be accomplished using H and N subtype specific primers in RT-PCR and real-time RT-PCR tests; or using sequence analysis of H and N genes. Subtype identification by these techniques is becoming increasingly common but is beyond the scope of many diagnostic laboratories not specialising in influenza viruses. Assistance is available from the WOAH Reference Laboratories and Collaborating Centres (see WOAH website for up-to-date list).

1.4. Assessment of pathogenicity

The term HPAI relates to the assessment of pathogenicity in chickens and implies the involvement of high pathogenicity strains of virus. It is used to describe a disease of fully susceptible chickens with clinical signs that may include one or more of the following: ocular and nasal discharges, coughing, snicking and dyspnoea, swelling of the sinuses and/or head, listlessness, reduced vocalisation, marked reduction in feed and water intake, cyanosis of the unfeathered skin, wattles and comb, incoordination, nervous signs and diarrhoea. In laying birds, additional clinical features include a marked drop in egg production usually accompanied by an increase in numbers of poor quality eggs. Typically, high morbidity is accompanied by high and rapidly escalating unexplained mortality. However, none of these signs can be considered pathognomonic and high mortality may occur in their absence. In addition, LPAI viruses that normally cause only mild or no clinical disease, may cause a much more severe disease if exacerbating infections or adverse environmental factors are present and, in certain circumstances, the spectrum of clinical signs may mimic HPAI.

The historical term 'fowl plague' has been abandoned in favour of the more accurate term HPAI. Because all naturally occurring HPAI viruses to date have been H5 and H7 subtypes and genomic studies have determined HPAI viruses arise by mutation of H5/H7 LPAI viruses, all H5/H7 LPAI viruses may potentially become HPAI but predicting which LPAI strains will mutate to HPAI is not possible. Pathogenicity shifts have been associated with changes to the proteolytic cleavage site of the haemagglutinin including: 1) substitutions of non-basic with basic amino acids (arginine or lysine); 2) insertions of multiple basic

amino acids from codons duplicated from the haemagglutinin cleavage site; 3) short inserts of basic and non-basic amino acids from unknown source; 4) recombination with inserts from other influenza A virus gene segments or avian host cellular genome (e.g. 28S rRNA) that lengthen the proteolytic cleavage site; and 5) loss of the shielding glycosylation site at residue 13 in combination with multiple basic amino acids at the cleavage site1. Amino acid sequencing of the cleavage sites of H5 and H7 subtype influenza A isolates of low pathogenicity for birds may identify viruses that have the capacity, following simple mutation, to have high pathogenicity for poultry.

The following criteria have been adopted by the WOAH for determining pathogenicity of an influenza A virus:

a) One of the two following methods to determine pathogenicity in chickens is used. A high pathogenicity influenza A virus is:

   i) any influenza A virus that is lethal[2] for six, seven or eight of eight 4- to 8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid

   or

   ii) any influenza A virus that has an intravenous pathogenicity index (IVPI) greater than 1.2. The following is the IVPI procedure:

   - Fresh infective allantoicfluid, confirmed free from APMV-1 and other extraneous agents, with a HA titre >1/16 (>24 or >log2 4 when expressed as the reciprocal) is diluted 1/10 in sterile isotonic saline.
   - 0.1 ml of the diluted virus is injected intravenously into each of ten 4- to 8-week-old SAN susceptible chickens; if possible, SPF chickens should be used.
   - Birds are examined at 24-hour intervals for 10 days. At each observation, each bird is scored 0 if normal, 1 if sick, 2 if severely sick, 3 if dead. (The judgement of sick and severely sick birds is a subjective clinical assessment. Normally, 'sick' birds would show one of the following signs and 'severely sick' more than one of the following signs: respiratory involvement, depression, diarrhoea, cyanosis of the exposed skin or wattles, oedema of the face and/or head, nervous signs. Dead individuals must be scored as 3 at each of the remaining daily observations after death[3].)
   - The IVPI is the mean score per bird per observation over the 10-day period. An index of 3.00 means that all birds died within 24 hours, and an index of 0.00 means that no bird showed any clinical sign during the 10-day observation period.

b) For all H5 and H7 viruses of low pathogenicity in chickens, the amino acid sequence of the connecting peptide of the haemagglutinin molecule (HA0) (i.e. the cleavage site) must be determined. The presence of several basic amino acids, inserts of cellular or viral nucleic acids or loss of specific glycosylation sites in the HA0 cleavage site is the genotypic standard for HPAI strains; therefore, if the isolate being tested has an HA0 cleavage site motif identical to previous HPAI viruses, it should be designated as HPAI irrespective of a low or high pathogenicity determined by pathotyping in chickens (see the table that lists all the reported haemagglutinin proteolytic cleavage sites of HA0 protein for H5 and H7 LPAI and HPAI viruses based on deduced amino acid sequence, which can be found on the OFFLU site (see footnote 2). Furthermore any isolate with a new motif must be tested *in vivo* by IVPI. In case of difficulties in the interpretation of the cleavage site motif, WOAH and/or FAO reference laboratories should be consulted.

   The WOAH classification system to identify influenza A viruses for which disease notification and control measures should be taken is defined in the *Terrestrial Code*.

   A variety of strategies and techniques have been used successfully to sequence the nucleotides at that portion of the HA gene coding for the cleavage site region of the haemagglutinin of H5 and

1 https://www.offlu.org/
2 When birds are too sick to eat or drink, they should be killed humanely.
3 When birds are too sick to eat or drink, they should be killed humanely and scored as dead at the next observation.

H7 subtypes of avian influenza virus, enabling the amino acids there to be deduced. This can be done by RNA extraction from the sample and direct sequencing of the haemagglutinin proteolytic cleavage site. Various stages in the procedure can be facilitated using commercially available kits and automated sequencers.

Determination of the cleavage site by sequencing or other methods has become the method of choice for initial assessment of the pathogenicity of these viruses and has been incorporated into agreed definitions. This has reduced the number of *in-vivo* tests, although the initial Sanger sequencing result of a HA cleavage site for an H5 or H7 LPAI virus should be confirmed by either inoculation of birds or deep sequencing using high throughput sequencing with a minimum of 1000 reads to to exclude the presence of any HPAI virus.

Although all the truly HPAI viruses isolated to date have been of H5 or H7 subtypes, at least three isolates, all of H10 subtype (H10N1, H10N4 and H10N5), have been reported that would have fulfilled both the WOAH and EU *in-vivo* definitions for HPAI viruses (Bonfante *et al.*, 2014; Wood *et al.*, 1996) as they killed 6/10, 7/10 and 8/10 chickens with IVPI values >1.2 when the birds were inoculated intravenously. However, these viruses did not induce death or signs of disease when inoculated intranasally and did not have a haemagglutinin cleavage site sequence compatible with HPAI virus. Similarly, other intravenously inoculated influenza A viruses are nephrotropic and birds that die have high titres of virus in their kidneys indicating a renal pathogenic mechanism (Slemons & Swayne, 1990), but such laboratory-induced pathobiology is not comparable to multi-organ infection and systemic disease caused by HPAI viruses. An H4N2 virus isolated from quail had a multibasic cleavage site sequence (PEKRRTR/GLF) but with an IVPI value of 0.0 (Wong et al., 2014) suggesting the multibasic cleavage site in viruses other than H5 and H7 alone may not be sufficient for declaration of HPAI virus and the in-vivo test should be carried out. Conversely, four viruses (A/chicken/Pennsylvania/1/83 [H5N2] and A/goose/Guangdong/2/96 [H5N1], A/turkey/England/87-92BFC/91 [H5N1] or A/chicken/Texas/298313/04 [H5N2]) have been described that have HA0 cleavage sites containing multiple basic amino acids, but which show low pathogenicity (IVPI <1.2) when inoculated intravenously into 6-week-old chickens (Londt *et al.*, 2007).No single explanation including the presence of a glycosylation site masking the HA0 cleavage site was reported emphasising both intra-haemagglutinin and multigenic influences in rare circumstances upon phenotypic expression of high pathogenicity. The presence of high pathogenicity haemagglutinin cleavage site in H5 and H7 influenza A viruses necessitates declaration of high pathogenicity to facilitate immediate control of the disease, otherwise a delay to complete *in-vivo* testing may result in continued onward transmission and spread between premises with severe consequence for future eradication once confirmed as a HPAI virus.

A table is available on the OFFLU website that lists all the reported haemagglutinin proteolytic cleavage site of HA0 protein for H5 and H7 LPAI and HPAI viruses based on deduced amino acid sequence. This table will be updated as new viruses are characterised; it can be found on the OFFLU site (see footnote 2).

1.5. Antigen capture and molecular techniques

At present, conventional virus isolation and characterisation techniques for the diagnosis of influenza A viruses remain a key method, for initial diagnosis of influenza A infection in a primary disease event and to provide virus for more detailed analyses including *in-vivo* testing and gene sequencing. Further they may be invaluable in confirming or disproving the presence of infectious virus when other test results Including conventional and real-time RT-PCR are all weakly positive. However, conventional methods tend to be costly, labour intensive and slow. There have been enormous developments and improvements in molecular and other diagnostic techniques, many of which are now routinely applied as a first choice for the diagnosis of influenza A infections.

1.5.1. Antigen detection

There are several commercially available AC-ELISA kits that can detect the presence of influenza A viruses in poultry (Swayne *et al.*, 2020). Most of the kits are enzyme immunoassays or are based on immunochromatography (lateral flow devices) and use a monoclonal antibody against the nucleoprotein; they should be able to detect any influenza A virus. The main advantage of these tests is that they can demonstrate the presence of influenza A within 15 minutes. The disadvantages are that they may lack sensitivity, they may not have been validated for different

species of birds, H and N subtype identification is not achieved and the kits are expensive. The tests should only be interpreted on a flock basis and not as an individual bird test. Oropharyngeal or tracheal samples from clinically affected or dead birds provide the best sensitivity. Nevertheless, the lack of sensitivity is a major drawback to the use of available antigen detection tests. Test sensitivities may vary between cloacal and tracheal swabs, whilst the tests can perform less well with samples from waterfowl or wild birds compared with chickens. Improved but moderate sensitivity in so named lateral flow devices was reported when using samples of feather follicles from birds infected with HPAI (Slomka *et al.*, 2012). Because of low sensitivity, antigen detection is mainly used for field screening of high mortality clinical cases for suspected influenza A virus infections followed by confirmation of results using a more sensitive laboratory-based test.

1.5.2. Direct RNA detection

As demonstrated by the current definitions of HPAI, molecular techniques are used preferentially for diagnosis for some time now. Furthermore, there have recently been developments towards their application to the detection and characterisation of influenza A viruses directly from clinical specimens of infected birds. It is imperative that when using highly sensitive molecular detection methods that allow rapid direct detection of viral RNA for confirmatory laboratory diagnosis of influenza A infections, stringent protocols are in place to prevent the risk of cross-contamination between clinical samples. In addition, RNA detection test methodologies should be validated to the WOAH standard (see Chapter 1.1.6 *Validation of diagnostic assays for infectious diseases of terrestrial animals*) using clinical material to demonstrate the tests as being 'fit for purpose' for application in a field diagnostic setting, which may include the use of internal test standards. The control reactions enable greater confidence in the integrity of the molecular reactions, clinical samples and results.

Furthermore, these evaluations enable the appropriate setting of test thresholds for interpretation between positive and negative samples. The increased sensitivity of real-time RT-PCR leads to the detection of viral RNA in samples in the absence of infectious virus and care should be taken when interpreting outputs with small detection limits that may not be indicative of active infection. This problem can be overcome, through the testing of multiple samples from the same cohort of infected birds, especially relevant when testing samples from domestic poultry for disease investigation.

In settings with more limited facilities, RT-PCR techniques on clinical samples can, with the correctly defined primers, result in rapid detection and subtype identification (at least of H5, H7 and H9 subtypes, and more recently developed assays are also available for other subtypes), including a cDNA product that can be used for nucleotide sequencing However, these approaches have now been largely replaced by the preferred molecular detection tests for influenza A virus by real-time RT-PCR, a modification to the RT-PCR that reduces the time for both identification of virus subtype and sequencing. For example, Spackman *et al.* (2002) used a single-step real-time RT-PCR primer/fluorogenic hydrolysis probe system to allow detection of influenza A viruses and determination of subtype H5 or H7. The test performed well relative to virus isolation and offered a cheaper and much more rapid alternative, with diagnosis on clinical samples in less than 3 hours. In additional studies, the real-time RT-PCR was shown to have sensitivity and specificity equivalent to virus isolation in numerous settings but updates to primer/probe design can be beneficial over time to accommodate genetic evolution in gene regions targeted by assays (Laconi *et al.*, 2020). These tests provide high sensitivity and specificity similar to those of virus isolation when used on tracheal and oropharyngeal swabs of chickens and turkeys, but may lack sensitivity for detection of influenza A virus in faecal swabs, faeces and tissues in some bird species, because of the presence of PCR inhibitors resulting in false-negative results (Das *et al.*, 2006). Incorporation of a positive internal control into the test will verify a proper test run. In addition, improvements in RNA extraction methods have been developed to eliminate most PCR inhibitors from test samples.

Real-time RT-PCR, usually based around the hydrolysis probe method for generation of the target-specific fluorescence signal, has become the method of choice in many laboratories for at least partial diagnosis directly from clinical specimens. The method offers rapid results, with sensitivity and specificity comparable to virus isolation. These are ideal qualities for influenza A outbreak management, where the period of time in which an unequivocal diagnosis can be obtained is crucial for decision making by the relevant Veterinary Authority. In addition, real-time

RT-PCR systems can be designed to operate in a 96-well format and combined with high-throughput robotic RNA extraction from specimens (Aguero *et al.*, 2007).

The approach to diagnosis using real-time RT-PCR adopted in most laboratories has been based on initial generic detection of influenza A virus in clinical samples, primarily by initially targeting the matrix (M) gene, which is highly conserved for all influenza A viruses, followed by specific real-time RT-PCR testing for H5 and H7 subtype viruses. Numerous assays have been reported for highly sensitive detection of M (or NP) gene fulfilling the criteria for a suitable screening test. For subtype identification, primers used in real-time RT-PCR are targeted at the HA2 region, as this is relatively well conserved within the haemagglutinin genes of the H5 and H7 subtypes (Spackman *et al.*, 2008; Spackman & Suarez, 2008). It has therefore served as the target region for these subtypes. Spackman *et al.* (2002) demonstrated specific detection of these subtypes, but cautioned that their H5 and H7 primer/probe sequences had been designed for the detection of North American H5 and H7 isolates and might not be suitable for all H5 and H7 isolates. This proved to be the case. Slomka *et al.* (2007) described modification of the H5 oligonucleotide sequences used by Spackman *et al.* (2002) to enable the detection of the Eurasian 'Goose/Guangdong lineage' (Gs/GD) H5N1 subtype and other Eurasian H5 subtypes that have been isolated within the past 15 years in both poultry and wild birds. As the group of 'Gs/GD' viruses diversified and spread across several continents it has become important that diagnostics in all settings have proven fit for purpose detection of this H5 lineage of viruses divided into multiple clades (World Health Organization/World Organisation for Animal Health/Food and Agriculture Organization & H5N1 Evolution Working Group, 2014). Newer rapid methods have been developed that enable simultaneous detection and subtyping speeding the time to achieve rapid identification of an influenza A virus using arrays (Hoffmann *et al.*, 2016) or microchip (Kwon *et al.*, 2019) technologies. The validated Eurasian real-time RT-PCR have proven valuable in the investigation of many H5Nx HPAI clinical samples and other subtypes submitted to International Reference Laboratories from Europe, Africa, Asia and North America since 2005 (Liu *et al.*, 2018; Slomka *et al.*, 2007). Each set of primers and probes needs to be validated against a diverse set of viruses to make the test applicable in a diverse range of avian species, and in viruses from broad geographic areas and time periods. In addition, real-time RT-PCR methods are now widely used for the rapid and accurate determination of the neuraminidase subtype (James *et al.*, 2018)

One of the problems with rapidly emerging new tests is that methods and protocols may be developed and reported without the test being properly validated. This has been addressed for some of the real-time RT-PCR protocols. In the European Union, National Reference Laboratories have collaborated to define and validate protocols that can be recommended for use within Europe (Hoffmann *et al.*, 2016; Nagy *et al.*, 2020; Slomka *et al.* 2007). Importantly this should include routine analysis of detected viruses (coordinated through WOAH Reference Laboratories) in standard assays to ensure reliable specific detection of contemporary viruses affecting poultry and other populations. In addition, given the high variability in the influenza A genome it is imperative that assays used in routine diagnosis and surveillance have ongoing demonstration of their fitness for detection of contemporary viruses validated for use in the region where they are applied. There should be an appropriate match for local strains taking account of significant regional and intercontinental variability amongst particular endemic viruses. Laconi *et al.* (2020) in reviewing five validated well used real-time RT-PCR methods concluded that continuous monitoring of assay performance using both in silico and *in-vitro* methodology was important as the emergence of new strains containing mutations within primer and probe binding areas might significantly affect the positive outcome of a test. Increasingly with improvements in assay design and using novel biochemical approaches screening assays relevant to all influenza A viruses from all hosts (animal and human) have been developed (Nagy *et al.*, 2020) with high relevance to an avian–'other' host interface.

Real-time RT-PCR protocols have been described that amplify regions across the cleavage site of the HA0 gene. This may result in useful tests for specific viruses. For example, Hoffman *et al.* (2007) have described a real-time RT-PCR test specific to the Eurasian HPAI H5N1 Qinghai-like clade 2.2 viruses that represents a rapid means of determining the pathotype for this subgroup of H5N1 HPAI viruses without sequencing. In situations where large numbers of positive samples/cases are detected during disease events, specific targeted real-time RT-PCR assays have been developed for the simultaneous sensitive detection and pathotyping of viruses. This can prove to be very useful, particularly when applying to early warning systems such as

surveillance of wild bird populations for local presence of HPAI (Graaf *et al.*, 2017; Naguib *et al.*, 2017).

Modifications to the straightforward RT-PCR method of detection of viral RNA have been designed to reduce the effect of inhibitory substances in the sample taken, the possibility of contaminating nucleic acids and the time taken to produce a result. The loop-mediated isothermal amplification (LAMP) system for H5 and H7 detection appears to show high sensitivity and reliable specificity (Ahn *et al.*, 2019; Bao *et al.*, 2014), but may have limited application because of susceptibility to viral mutations affecting the target regions, reducing virus detection (Postel *et al.*, 2010).

Increasing innovation and technological improvements have made it possible that molecular based and improved antigen detection technologies have developed sufficiently to permit rapid flock side tests for the detection of presence of influenza A virus specific subtypes and pathogenicity markers (Inui *et al.*, 2019). Furthermore, innovations in test design have enabled for example the development of point of care chip based ultrafast PCR approaches (Kwon *et al.*, 2018) with increasing application anticipated in the future.

1.5.3. Gene sequencing

Currently real-time RT-PCR is the preferred method of virus surveillance because the test provides rapid sensitive diagnostics for influenza H5, H7 and H9 and is available in high throughputs. However, greater use of sequencing technologies particularly as unit costs reduce with improvement in technology, offer powerful opportunities to simultaneously detect and sequence from clinical samples in a laboratory or field setting, for example applying nanopore technology (King *et al.*, 2020).

Increasingly gene sequencing is being applied not only to detailed characterisation of viruses for use in molecular epidemiology but also in virus subtyping and defining markers for host range including zoonotic risk. Sanger sequencing methodology has been widely used for decades and enables the rapid determination of typically a single (H) target gene in 24-36 hours to define virus pathogenicity (see Section B.1.1.1) and still has widespread utility. However, as genomic data can be rapidly determined using next generation sequencing technology it enables a broader analysis using a range of bioinformatics tools (Zhang *et al.*, 2017). For example, with the advent of greater access to sequencing methodology either through specialised laboratories or commercial providers it is now possible to determine the genomic sequences of influenza A viruses from birds to provide a level of characterisation important in rapid pathogen identification and outbreak intervention. Conventionally nucleotide sequences have been used in outbreak epidemiology to infer virus origin and precise relationships between different viruses associated within the same event (by phylogeny) to support outbreak management. Virus gene sequences of haemagglutinin and neuraminidase can rapidly be compared to known sequences of all subtypes in gene databases and used to reveal closest match thereby identifying the virus subtype and phylogenetic relationships. This often avoids the need to culture the virus for rapid identification although reliability and quality of data reduces with increasing cycle threshold values in samples from real-time RT-PCR testing.

Increasingly such analyses are now being applied at the whole genome level to reveal virus genotypes and provide greater analytical specificity to the analyses. Such approaches are especially valuable to track since virus evolution which can be more precisely mapped including change through genetic reassortment, a key mechanism associated with virus diversity and fitness for birds. This approach is especially valuable for early or first incursions in a new event as it enables greater precision in determining virus origin and the mechanisms leading to the emergence of virus. This has become increasingly important in characterising the rapid evolution and wide diversity of Gs/GD lineage viruses associated with transcontinental spread. Translation of nucleotide sequences of all genomic segments into amino acid sequences enables data mining for other virus characteristics or traits such as tropism, host range markers including zoonotic and predicted antiviral drug susceptibility which are invaluable for informing outbreak management.

2. Serological tests

2.1. Enzyme-linked immunoassay (ELISA)

Commercial ELISA kits that detect antibodies against the nucleoprotein are available. Kits with an indirect and competitive/blocking format have been developed and validated, and are now being used to detect influenza A virus-specific antibodies. Several avian influenza competitive ELISA (AIV C-ELISA) or blocking ELISA (AIV B-ELISA) have been developed and validated as a more sensitive alternative to the AGID test for the detection of influenza A group reactive antibodies in sera from chickens and other bird species (SCAHLS, 2009). This AIV ELISA platform, as either a "competitive" or "blocking" format, detects antibodies to influenza A viruses by allowing these antibodies to compete for antigen binding sites with a monoclonal antibody against an epitope on the nucleoprotein that is conserved in all influenza A viruses.

The kits should be validated for the specific species of interest and for the specific purpose(s) for which they are to be used. Several different test and antigen preparation methods are used. Such tests have usually been evaluated and validated by the manufacturer, and it is therefore important that the instructions specified for their use be followed carefully. Please see the WOAH Register for kits certified by the WOAH[4]. ELISA kits are of moderate cost and are amenable to high throughput screening for influenza A virus infections and have strong utility for application to large-scale serosurveillance programmes and compare favourably to HI (Arnold *et al.*, 2018). However, all positive results must be followed by HI test for subtyping to H5 and H7. Some subtype-specific ELISA kits are available, e.g. for antibodies to H5, H7, H9 and some N subtypes i.e. N1 but generally are of lower sensitivity than influenza A ELISA.

2.2. Agar gel immunodiffusion

All influenza A viruses have antigenically similar nucleoprotein and antigenically similar matrix antigens. Owing to this fact AGID tests are able to detect the presence or absence of antibodies to any influenza A virus. Concentrated virus preparations, as described above, contain both matrix and nucleoprotein antigens; the matrix antigen diffuses more rapidly than the nucleoprotein antigen. AGID tests have been widely and routinely used to detect specific antibodies in chicken and turkey flocks as an indication of infection, but AGID tests are less reliable at detecting antibodies following infection with influenza A viruses in other avian species. These have generally employed nucleoprotein-enriched preparations made from the chorioallantoic membranes of embryonated chicken eggs (Beard, 1970) that have been infected at 10 days of age, homogenised, freeze–thawed three times, and centrifuged at 1000 ***g***. The supernatant fluids are inactivated by the addition of 0.1% formalin or 1% betapropiolactone, recentrifuged and used as antigen. Not all avian species may produce precipitating antibodies following infection with influenza viruses, for example ducks. The AGID is a low-cost serological screening test of reduced sensitivity for detection of generic influenza A infections, but must be followed by HI tests for subtyping influenza A positives as to H5 and H7.

Tests are usually carried out using gels of 1% (w/v) agarose or purified type II agar and 8% (w/v) NaCl in 0.01 M phosphate buffer, pH 7.2, poured to a thickness of 2–3 mm in Petri dishes or on microscope slides, and incubated in a humidified chamber. Using a template and cutter, wells of approximately 5 mm in diameter are cut into the agar at a distance of about 3 mm from each other. A pattern of wells must place each suspect serum adjacent to a known positive serum and antigen. Each well should have reagent added to fill the well, corresponding to the top of the meniscus with the top of the gel, but do not over fill. Approximately 25–30 µl of each reagent should be required per well, but this depends on thickness of the gel, with thicker gels requiring an additional volume of reagent.

Wells should be examined for precipitin lines at 24 hours, and weak positive samples or samples for which specific lines have not formed should be incubated longer and examined again at 48 hours. The time to formation of visible precipitin line is dependent on the concentrations of the antibody and the antigen. The precipitin lines are best observed against a dark background that is illuminated from behind. A specific, positive result is recorded when the precipitin line between the known positive control wells is

4 https://www.woah.org/en/what-we-offer/veterinary-products/#ui-id-5/

continuous with the line between the antigen and the test well. Crossed lines are interpreted to be caused by the test serum lacking identity with the antibodies in the positive control well.

Whilst the AGID is relatively inexpensive and suitable for resource limited settings it is being increasingly replaced by other platforms such as ELISA for flock level serological investigations including pre export/import screening of birds for historical exposure to influenza A.

2.3. Haemagglutination and haemagglutination inhibition tests

Variations in the procedures for HA and HI tests are practised in different laboratories. The following recommended examples apply to the use of V-bottomed microwell plastic plates in which the final volume for both types of test is 0.075 ml. U- bottomed plates can be used but care in reading is required as the clarity is less defined. The reagents required for these tests are isotonic PBS (0.01 M), pH 7.0–7.4, and red blood cells (RBCs) taken from a minimum of three SPF or SAN chickens and pooled into an equal volume of Alsever's solution. Cells should be washed three times in PBS before use as a 1% (packed cell v/v) suspension. Positive and negative control antigens and antisera should be run with each test, as appropriate.

2.3.1. Haemagglutination test

i) Dispense 0.025 ml of PBS into each well of a plastic V-bottomed microtitre plate.

ii) Place 0.025 ml of virus suspension (i.e. infective allantoic fluid) in the first well. For accurate determination of the HA content, this should be done from a close range of an initial series of dilutions, i.e. 1/3, 1/4, 1/5, 1/6, etc.

iii) Make twofold dilutions of 0.025 ml volumes of the virus suspension across the plate.

iv) Dispense a further 0.025 ml of PBS to each well.

v) Dispense 0.025 ml of 1% (v/v) chicken RBCs to each well.

vi) Mix by tapping the plate gently and then allow the RBCs to settle for about 40 minutes at room temperature, i.e. about 20°C, or for 60 minutes at 4°C, if ambient temperatures are high, by which time control RBCs should have formed a distinct button.

vii) HA is determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration should be read to the highest dilution giving complete HA (no streaming); this represents 1 HA unit (HAU) and can be calculated accurately from the initial range of dilutions.

2.3.2. Haemagglutination inhibition test

i) Dispense 0.025 ml of PBS into each well of a plastic V-bottomed microtitre plate.

ii) Place 0.025 ml of serum into the first well of the plate.

iii) Make twofold dilutions of 0.025 ml volumes of the serum across the plate.

iv) Add 4 HAU of virus/antigen in 0.025 ml to each well and leave for a minimum of 30 minutes at room temperature (i.e. about 20°C) or 60 minutes at 4°C.

v) Add 0.025 ml of 1% (v/v) chicken RBCs to each well and mix gently, allow the RBCs to settle for about 40 minutes at room temperature, i.e. about 20°C, or for 60 minutes at 4°C if ambient temperatures are high, by which time control RBCs should have formed a distinct button.

vi) The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) should be considered to show inhibition.

vii) The validity of results should be assessed against a negative control serum, which should not give a titre >1/4 (>2^2 or >$\log_2$ 2 when expressed as the reciprocal), and a positive control serum for which the titre should be within one dilution of the known titre.

The HI test is primarily used to determine if antibodies indicating influenza A virus infections are subtyped as H5 and H7 or other H subtypes (H1-4, H6, H8-16). HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 (2^4 or $\log_2$ 4 when expressed as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titre is 1/8 (2^3 or $\log_2$ 3) or more. The meaning of a minimum positive titre should not be misinterpreted; it does not imply, for example, that immunised birds with that titre will be protected against challenge or that birds with lower titres will be susceptible to challenge. Appropriate virus/antigen control, positive control serum and RBC control well should be included with each batch of HI tests.

Chicken sera rarely give nonspecific positive agglutination reactions in this test and any pretreatment of the sera is unnecessary. Sera from species other than chickens may sometimes cause agglutination of chicken RBCs resulting in nonspecific agglutination. Therefore, each serum should first be tested for this idiosyncrasy and, if present, it should be inhibited by adsorption of the serum with chicken RBCs. This is done by adding 0.025 ml of packed chicken RBCs to each 0.5 ml of antisera, mixing gently and leaving for at least 30 minutes; the RBCs are then pelleted by centrifugation at 800 *g* for 2–5 minutes and the adsorbed sera are decanted. Alternatively, RBCs of the avian species under investigation could be used. Nonspecific inhibition of agglutination can be caused by steric inhibition when the tested serum contains antibodies against the same N subtype as the H antigen used in the HI test. The steric inhibition reaction can result in RBC buttoning in the bottom of the plate or streaming at the same rate as the control. If using whole virus antigen in HI test for subtyping, it is important to ensure that two antigens for each haemagglutinin subtype are used with heterologous neuraminidase i.e. H5N1 and H5N6 to eliminate the possibility of interference in the assay with anti N antibodies that can lead to false typing results. Alternatively the H antigen used can be recombinant or purified H protein that lacks N protein. The HI test is based on antigenic binding between the H antigen and antisera and thus other factors may cause nonspecific binding of the H antigen and sera leading to a nonspecific inhibition reaction. At this time there are no documented cross reactions or nonspecific inhibition reactions between the different haemagglutinin subtypes of influenza A.

2.4. Neuraminidase inhibition test

The neuraminidase-inhibition test has been used to identify the influenza A neuraminidase type of isolates as well as to characterise the antibody in infected birds. The procedure requires specialised expertise and reagents; consequently, this testing is usually done in a WOAH Reference Laboratory. The DIVA (differentiating infected from vaccinated animals) strategy used previously in Italy also relies on a serological test to detect specific anti-N antibodies; the test procedure has been described (Capua *et al.*, 2003).

C. REQUIREMENTS FOR VACCINES

1. Background

Vaccination alone is not the solution to the control of HPAI if eradication is the desired result. Without the application of monitoring systems, strict biosecurity and depopulation in the face of infection, HPAI will become endemic in vaccinated poultry populations. Long-term circulation of the virus in a vaccinated population may result in both antigenic and genetic changes as has occurred with H5Nx (Gs/GD lineage), H7N3, H7N9 and H9N2 influenza A viruses in Mexico, and various Middle Eastern and Asian countries (Swayne & Sims, 2020). Currently used vaccines and the use of vaccination have been reviewed (FAO, 2016; Swayne & Sims, 2020). The haemagglutinin is the primary influenza A viral protein that elicits a protective immune response used in officially approved poultry vaccines and such immunity is haemagglutinin subtype specific.

To date, the majority of influenza A vaccines used in poultry have been inactivated whole virus vaccines prepared from infective allantoic fluid of embryonated chicken eggs, inactivated by beta-propiolactone or formalin and emulsified with mineral oil adjuvants. Because of the potential for reassortment leading to increased virulence, live conventional influenza vaccines against any subtype are not recommended. However, biotechnology holds great potential to generate live avian influenza virus vaccines with altered gene segments which reduce the risk of reassortment, limit replication and abrogate negative aspects of live influenza A virus vaccines (Song *et al.*, 2007). The existence of a large number of haemagglutinin subtypes (i.e. H1–16), together with the known variation of different strains within a subtype, pose serious problems when selecting strains to produce inactivated influenza A

vaccines. In addition, some isolates do not grow to a sufficiently high titre to produce adequately potent vaccines without costly pre-concentration. While some vaccination strategies use autogenous vaccines, i.e. vaccines prepared from isolates specifically involved in an epizootic, others rely on vaccines prepared from biologically characterised, fully approved seed strain viruses possessing the same haemagglutinin subtype as the field virus and capable of yielding high concentrations of antigen. Historically, inactivated vaccines for LPAI and HPAI control have used LPAI viruses with a matching haemagglutinin subtype from outbreaks as seed strains, but with development of resistance in the field associated with prolonged vaccine use, the majority of inactivated vaccine seed strains are now reverse genetic derived virus with antigenically close matching haemagglutinin, and sometimes neuraminidase, to circulating field viruses. Use of HPAI viruses as inactivated vaccine seed strains is strongly discouraged because of biosafety concerns.

Since the 1970s in the USA, inactivated influenza A vaccines have been used primarily in turkeys against LPAI viruses under emergency vaccination programs, but since the 2000s, most vaccines have been against H1 and H3 swine influenza A viruses used under a routine preventative vaccination program in breeder turkeys (Swayne *et al.*, 2020). Since the early 1990s, vaccination against H9N2 LPAI virus has been used extensively in Asia and the Middle East using billions of inactivated vaccine doses (Swayne & Sims, 2020). Vaccination against HPAI was first used in Mexico during the H5N2 outbreaks of 1994–1995 (Villarreal, 2007), and in Pakistan (Naeem, 1998) during the H7N3 outbreaks of 1995. Beginning with H5N1 goose/Guangdong (Gs/GD) lineage HPAI outbreaks in Hong Kong in 2002 (Sims, 2003), a vaccination policy was adopted using H5N2 LPAI vaccine seed strains and subsequently replaced with H5Nx reverse genetic vaccine seed strains, as the field virus spread throughout and outside of China Between 2002 and 2010, 113 billion doses of vaccine was used to control HPAI with 95% being inactivated and 5% recombinant vaccines, and a similar usage rate continues (Swayne *et al.*, 2011; Swayne & Sims, 2020). As the H5Nx Gs/GD lineage HPAI spread across the global, additional countries have implemented emergency and/or preventative vaccination programs for HPAI control. Similarly, preventive vaccination against H5N1 HPAI has been permitted for outdoor poultry and zoo birds in several European Union countries in in the 2000s.

Live recombinant virus-vectored vaccines with H5 influenza A virus haemagglutinin gene inserts have been approved and used in a few countries since 1997, mostly in chickens, and include recombinant fowl poxvirus (rFPV), recombinant Newcastle disease virus (rNDV) and recombinant herpesvirus turkey vaccines (rHVT). Since 2015, non-replicating, haemagglutinin based H5 RNA particle, H5 expressed baculovirus and H5 DNA vaccines have been approved for poultry but have had limited use (Swayne & Sims, 2020).

1.1. Rationale and intended use of the product

Experimental work has shown, for HPAI and LPAI, that potent and properly administered vaccines increase resistance to, or prevent infection, protect against clinical signs and mortality, prevent drops in egg production, reduce virus shedding from respiratory and intestinal tracts, protect from diverse field viruses within the same haemagglutinin subtype, protect from low and high challenge exposure, and reduce excretion and thus prevent contact transmission of challenge virus (Capua *et al.*, 2004; Swayne & Sims, 2020). Although, in experimental vaccination studies, a challenge virus is still able to infect and replicate in clinically healthy vaccinated SPF birds when exposed to high doses, the quantities shed may be insufficient for onward transmission of the virus (Van der Goot et al., 2005). Most of the work evaluating vaccines has been done in chickens and turkeys and some care must be taken in extrapolating the results obtained to other species. Most national HPAI and LPAI control regulations reserve the right to use vaccines in emergencies.

2. Outline of production and minimum requirements for conventional vaccines

The information below is based primarily on the experiences in the USA and the guidance and policy for regulatory approval of influenza A vaccines in that country (United States Department of Agriculture, 1995 [updated 2006]). The basic principles for producing vaccines, particularly inactivated vaccines, are common to several viruses e.g. Newcastle disease (chapter 3.3.10).

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.8 *Principles of veterinary vaccine production.* The guidelines given here and in chapter 1.1.8 are intended to be general in nature and may be supplemented by national and regional requirements.

The vaccine production facility should operate under the appropriate biosecurity procedures and practices. If HPAI virus is to be used in challenge studies, the facility used for such studies should meet the competent veterinary authority within the country minimum requirements for Containment Group 3 pathogens as outlined in chapter 1.1.4.

2.1. Characteristics of the seed

2.1.1. Biological characteristics

For any subtype, only well characterised influenza A virus of proven low pathogenicity, preferably obtained from an international or national repository, should be used to establish a master seed for inactivated vaccines. HPAI viruses should not be used as seed virus for vaccine. For HPAI, reverse genetic produced vaccine seed strains based on haemagglutinin gene of the HPAI virus are preferred, but should have the cleavage site sequence altered to that of a H5/H7 LPAI virus.

A master seed is established from which a working seed is obtained. The master seed and working seed are produced in SPF or SAN embryonated eggs. The establishment of a master culture may only involve producing a large volume of infective allantoic fluid (minimum 100 ml), which can be stored as lyophilised aliquots (0.5 ml).

2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)

The established master seed should be controlled/examined for sterility, safety, potency and absence of specified extraneous agents.

2.2. Method of manufacture

2.2.1. Procedure

For vaccine production, a working seed, from which batches of vaccine are produced, is first established in SPF or SAN embryonated eggs by expansion of an aliquot of master seed to a sufficient volume to allow vaccine production for 12–18 months. It is best to store the working seed in liquid form at below –60°C as lyophilised virus does not always multiply to high titre on subsequent first passage.

The routine procedure is to dilute the working seed in sterile isotonic buffer (e.g. PBS, pH 7.2), so that about 10^3–10^4 EID_{50} in 0.1 ml are inoculated into each allantoic cavity of 9- to 11-day-old embryonated SPF or SAN chicken eggs. These are then incubated at 37°C. Eggs containing embryos that die within 24 hours should be discarded. The incubation time will depend on the virus strain being used and will be predetermined to ensure maximum yield with the minimum number of embryo deaths.

The infected eggs should be chilled at 4°C before being harvested. The tops of the eggs are removed and the allantoic fluids collected by suction. The inclusion of any yolk material and albumin should be avoided. All fluids should be stored immediately at 4°C and tested for bacterial contamination.

In the manufacture of inactivated vaccines, the harvested allantoic fluid is treated with either formaldehyde (a typical final concentration is 1/1000, i.e. 0.1% formalin) or beta-propiolactone (BPL) (a typical final concentration is 1/1000–1/4000, i.e. 0.1–0.025% of 99% pure BPL). The time required must be sufficient to ensure freedom from live virus. Most inactivated vaccines are formulated with non-concentrated inactivated allantoic fluid (active ingredient). However, active ingredients may be concentrated for easier storage of antigen. The active ingredient is usually emulsified with mineral or vegetable oil and surfactants. The exact formulations are generally commercial secrets.

2.2.2. Requirements for substrates and media

The inactivated influenza A vaccines prepared from conventional virus are produced in 9- to 11-day-old embryonated SPF or SAN chicken eggs. The method of production is basically the same as for propagating the virus aseptically; all procedures are performed under sterile conditions.

2.2.3. In-process controls

For inactivated vaccines, completion of the inactivation process should be tested in embryonated eggs, taking at least 10 aliquots of 0.2 ml from each batch and passaging each aliquot at least twice through SPF or SAN embryos. Viral infectivity must not remain.

2.2.4. Final product batch tests

Most countries have published specifications for the control of production and testing of vaccines, which include the definition of the obligatory tests on vaccines during and after manufacture.

i) Sterility and purity

Tests for sterility and freedom from contamination of biological materials intended for veterinary use may be found in chapter 1.1.9.

ii) Safety

For inactivated vaccines, a double dose is administered by the recommended route to ten 3-week-old birds, and these are observed for 2 weeks for absence of clinical signs of disease or local lesions.

iii) Batch potency

Potency of influenza A vaccine is generally evaluated by testing the ability of the vaccine to induce a significant HI titre in SPF or SAN birds. Conventional potency testing involving the use of three diluted doses and challenge with HPAI virus (e.g. chapter 3.3.10) may also be used for vaccines prepared to give protection against LPAI subtypes. For inactivated vaccines against HPAI or LPAI virus, potency tests may rely on the measurement of immune response or challenge and assessment of morbidity, mortality (HPAI only) and quantitative reduction in challenge virus replication in respiratory (oropharyngeal or tracheal) and intestinal (cloaca) tracts. Assessment of haemagglutinin antigen content could allow for *in-vitro* extrapolation to potency for subsequent vaccine batches.

iv) Preservatives

A preservative may be used for vaccine in multidose containers.

2.3. Requirements for regulatory approval

2.3.1. Safety requirements

i) Target and non-target animal safety

Most inactivated influenza A vaccines are approved for use in chickens and turkeys. Field trials in the target species should be conducted to determine tolerance and safety of the vaccine at full dose. Recently the use of inactivated influenza A vaccines has been expanded to ducks, geese, other poultry and zoo birds. Any extra-label use of the vaccines should be done cautiously and under the supervision of a veterinarian experienced in disease control through vaccination in the test species. Care must be taken to avoid self-injection with oil emulsion vaccines.

ii) Reversion-to-virulence for attenuated/live vaccines

Only inactivated influenza A virus vaccines are recommended. Live conventional influenza vaccines against any subtype are not recommended because of the risk for reassortment of gene segments of vaccine virus with field virus, potentially creating more pathogenic field viruses.

iii) Environmental consideration

None

2.3.2. Efficacy requirements

i) For animal production

For regulatory purposes, influenza A vaccines should pass an efficacy challenge test using a statistically relevant number of SPF or SAN chickens per group. The challenge should occur at a minimum of three weeks post-vaccination, using a challenge HPAI virus dose that

causes 90% or greater mortality in the sham population. A standardised challenge dose of 10^6 mean chicken embryo infectious doses is most widely used. Protection from mortality in the vaccine group should be a minimum of 80%. For LPAI, mortality is not a feature of challenge models, therefore a statistically significant reduction in virus shedding titre and/or the number of birds shedding virus from oropharynx or cloaca should be observed between sham and test vaccine groups. Other metrics of protection can be used to determine efficacy such as prevention of drops in egg production.

In establishing minimum antigen requirements, 50 PD_{50} or 3 µg of haemagglutinin per dose have been recommended (Swayne & Sims, 2020). Minimum HI serological titres in field birds should be 1/32 to protect from mortality or greater than 1/128 to provide reduction in challenge virus replication and shedding for antigenically close related vaccine and challenge viruses.

ii) For control and eradication

Efficacy should be the same as for animal production.

2.3.3. Stability

When stored under the recommended conditions, the final vaccine product should maintain its potency for at least 1 year. Inactivated vaccines must not be frozen.

3. Vaccines based on biotechnology

3.1. Vaccines available and their advantages

Recombinant live vaccines for influenza A viruses have been produced by inserting the gene coding for the influenza A virus haemagglutinin into a non-influenza live virus vector and using this recombinant virus to immunise poultry against influenza A (Swayne & Sims, 2020). Recombinant live vector vaccines have several advantages over inactivated influenza A vaccines: 1) they induce mucosal, humoral and cellular immunity; 2) they can be mass administered *in ovo* or to 1-day-old birds in the biosecure hatchery to induce early protection; and 3) they enable easy serological differentiation of infected from non-infected vaccinated birds because they do not induce the production of antibodies against the nucleoprotein or matrix antigens that are common to all influenza A viruses; i.e. differentiation of infected from vaccinated (DIVA) animals. Therefore, only field-infected birds will exhibit antibodies in the AGID test or ELISAs directed towards the detection of influenza group A (nucleoprotein and/or matrix) antibodies. However, recombinant live vaccines have limitations in that they may have reduced replication and thus induce no or only partial protective immunity in birds that have had field exposure to or vaccine induced immunity against the vector virus or the H gene insert (Bertran *et al.*, 2018; Swayne & Sims, 2020). If used in day-old or young birds, the effect of maternal antibodies to the vector virus on vaccine efficacy may vary with the vector type; i.e. most severe inhibition in decreasing order for Newcastle disease virus, fowl poxvirus and HVT vectors. In addition, because the vectors are live viruses that may have a restricted host range, the use of such vaccines must be restricted to species in which efficacy has been demonstrated.

A rFPV-H5 vaccine, with H gene insert for A/turkey/Ireland/1378/1983 (H5N8), was developed in the early 1980s and authorised beginning in 1998 for use against H5N2 LPAI of Mexico (Swayne & Sims, 2020). This vaccine has principally been used in Mexico with expansion into several other countries within Central America and Vietnam with over 9 billion doses used between 1998 and 2016. This rFPV-H5 has had the H gene insert updated to A/chicken/Mexico/P-14/2016 (H5N2) (Bertran *et al.*, 2020). An rFPV-H7 with haemagglutinin insert from A/chicken/Guanajuato/07437-15/2015 (H7N3) has been developed and approved with deployment to Mexico in 2018 against H7N3 HPAI, and a rFPV-H5 with H and N gene inserts from A/goose/Guangdong/1996 (H5N1, clade 0) was used in China against the H5N1 HPAI during 2005 (Chen & Bu, 2009; Criado *et al.*, 2019; Swayne & Sims, 2020). rFPV can be effective when given to 1-day-old chicks with varying levels of maternal immunity (Arriola *et al.*, 1999). However, when very high levels of inhibitory immunity is anticipated because of previous infection or vaccination, the efficacy of the recombinant live vaccine in such day-old chicks should be confirmed and may require a prime-boost application of recombinant vaccine followed at a minimum 10 days later by inactivated influenza A vaccine boost to give optimal immunity (Richard-Mazet *et al.*, 2014; Swayne & Sims, 2020).

Newcastle disease virus can also be used as a vector for expressing influenza haemagglutinin genes. A recombinant Newcastle disease vaccine virus (rNDV) expressing a H5 HA gene (rNDV-H5) was shown to protect SPF chickens against challenge with both virulent Newcastle disease virus and a HPAI H5N2 virus (Veits *et al.*, 2006). A similar recombinant virus based on Newcastle disease virus vaccine strain La Sota and expressing H gene of A/goose/Guangdong/1996 (clade 0)(H5N1) was produced in China (the People's Rep. of) (Ge *et al.*, 2007) and reported to be efficacious in protection studies with either virus. This rNDV-H5 (clade 0) vaccine has been used widely with subsequent updating of HA insert twice with clade 2.3.4 and 2.3.2 clade haemagglutinin inserts (Swayne & Sims, 2020). An rNDV-H5 with H gene insert from A/chicken/Mexico/435/2005 (H5N2) has been developed, approved and deployed in Mexico against H5N2 LPAI (Swayne & Sims, 2020). An rNDV-H5 vaccine with H gene insert from A/chicken/Iowa/04-20/2015 (H5N2) (Gs/GD lineage, clade 2.3.4.4) insert was effective in protecting chickens against challenge with homologous H5N2 HPAI virus in chickens lacking immunity to the Newcastle disease virus vector or the H gene insert, but rNDV-H5 vaccine was ineffective as a primary or booster vaccine in poultry with maternal immunity or well-immunised against Newcastle disease or the H5 haemagglutinin protein (Bertran *et al.*, 2018). rNDV-H5 vaccines are effective as a primary vaccine if used in Newcastle disease or H5 antibody negative chickens, or as a priming vaccine followed by a boost with an inactivated influenza A vaccine in Newcastle disease or H5 antibody positive chickens. The major advantage of rNDV-H5 is the ability for low cost mass application by spray in the hatchery or field (Swayne & Sims, 2020).

Since 2010, a rHVT-H5 with haemagglutinin insert of A/swan/Hungary/4999/2006 (Gs/GD lineage, clade 2.2) has been approved and used in Egypt and Bangladesh against H5Nx Gs/GD lineage HPAI and in Mexico against H5N2 LPAI (Rauw *et al.*, 2011; Swayne & Sims, 2020). This rHVT-H5 vaccine has produced broad protection across diverse H5 HPAI viruses (Rauw *et al.*, 2011). Furthermore, maternally derived antibodies to rHVT vector or H5 haemagglutinin protein have had minimal negative impact on the effectiveness of the vaccine in broiler chickens after a single vaccination at 1 day of age (Bertran *et al.*, 2018). The rHVT-H5 is limited to application only *in ovo* or at 1 day of age to chickens in the hatchery, as application later on the farm is not feasible because of the ubiquitous infection by Marek's disease viruses or use of Marek's disease vaccines.

Because of the induction of broader immunity across mucosal, humoral and cellular areas, recombinant live vectored vaccines have had a longer use life in the field before appearance of field viruses that are resistant to the vaccine strains as compared to inactivated whole virus vaccines which produce primarily a strong humoral immunity. A recombinant duck enteritis virus in domestic ducks has been developed and shown efficacy but is pending regulatory approval and deployment in China (People's Rep. of) (Liu *et al.*, 2011).

Non-replicating haemagglutinin-based RNA particle and DNA vaccines with H gene from A/Gyrfalcon/Washington/40188-6/2014 (H5N8) (Gs/GD lineage, clade 2.3.4.4) have been approved for poultry use in the USA (Swayne & Sims, 2020). The H5 RNA particle vaccine is part of the USA emergency vaccine bank, along with rHVT-H5 and an inactivated H5N2 vaccines. The H5 RNA particle vaccine has been demonstrated to be an effective booster vaccine to replace rg inactivated H5Nx vaccine (Bertran *et al.*, 2017). A baculovirus with H gene insert from A/duck/China/E319-2/2003 (Gs/GD lineage, clade 2.3.3) has been approved for poultry use in Bangladesh, Egypt and Mexico (Swayne & Sims, 2020). Since this category of vaccine only contain the specific influenza A haemagglutinin protein, they are easily amenable to serological DIVA testing using assays designed for identifying antibodies to the nucleoprotein/matrix protein. However, field reports of protection with vectored and conventional influenza A vaccines suggest that protection by single dose of the vectored vaccines for long lived poultry is not feasible, with long-term field protection requiring a booster with inactivated influenza A vaccine or non-replicating, haemagglutinin-based vaccine (Swayne & Sims, 2020).

In addition to these approved vaccines, various experimental haemagglutinin-based H5 and H7 influenza A vaccines have been described using *in-vivo* or *in-vitro* expression systems including recombinant adenoviruses, salmonella, vaccinia, avian leucosis virus, various eukaryotic systems (plants or cell cultures) and infectious laryngotracheitis virus (Swayne & Sims, 2020).

3.2. Special requirements for biotechnological vaccines, if any

Live recombinant vectored vaccines with influenza A haemagglutinin gene inserts should have an environmental impact assessment completed to determine the risk of the vaccine to be virulent in non-target avian species and will not increase in virulence in the target avian species.

4. Surveillance methods for detecting infection in vaccinated flocks and vaccinated birds

A strategy that allows differentiation of infected from vaccinated animals (DIVA), has been put forward as a possible solution to the eventual eradication of HPAI and H5/H7 LPAI without involving mass culling of birds and the resulting economic damage, especially in developing countries (FAO, 2004). This strategy has the benefits of vaccination (less virus in the environment), but the ability to identify infected flocks would still allow the implementation of additional control measures, including stamping out of infected flocks. DIVA strategies use one of two broad detection schemes within the vaccinated population: 1) detection of influenza A virus ('virus DIVA'), or 2) detection of antibodies against influenza A field virus infection ('serological DIVA'). At the flock level, a simple method consists of regularly monitoring sentinel birds left unvaccinated in each vaccinated flock, but this approach does have some management problems, particularly with regards to identifying the sentinels in large flocks. As an alternative or adjunct system, testing for field exposure may be performed on the vaccinated birds either by detection of field virus or antibodies against the virus. To detect the field virus, oropharyngeal or cloacal swabs from baseline daily mortality or sick birds can be tested, individually or as pools, by molecular methods, such as real-time RT-PCR or AC-ELISA of the vaccinated populations (Swayne & Kapczynski, 2008).

To use serological DIVA schemes, vaccination systems that enable the detection of field exposure in vaccinated populations should be used. Several systems have been used. First, use of a vaccine containing a virus of the same haemagglutinin subtype but a different neuraminidase (N) from the field virus. Antibodies to the N of the field virus act as natural markers of infection. This system was used in Italy following the re-emergence of a H7N1 LPAI virus in 2000, and used an H7N3 inactivated vaccine with the detection of N3 antibodies indicating a vaccinated flock, N1 antibodies indicating infection, and both N1 and N3 antibodies indicating an infected, vaccinated flock (Capua *et al.*, 2003). Problems with this system would arise if a field virus emerges that has a different N antigen to the existing field virus or if subtypes with different N antigens are already circulating in the field as is present in many low and middle income countries with H5Nx (Gs/GD lineage), H9N2 and other NA subtypes in live poultry markets (Swayne & Sims, 2020). A second serological DIVA option is the use of vaccines that contain only HA, e.g. replicating or non-replicating recombinant vaccines, which allows validated, classical AGID and nucleoprotein (NP)- or matrix protein-based ELISAs to be used to detect antibodies indicative of infection in vaccinated birds. Finally, for inactivated vaccines, a test that detects antibodies to the nonstructural viral or M2e proteins have been described (Avellaneda *et al.*, 2010; Lambrecht *et al.*, 2007). These systems are yet to be validated in the field.

5. Continued evaluation and updating of vaccine seed strains to protect against emergent variant field virus strains

Historically, H5 LPAI inactivated vaccine seed strains and recombinant fowl poxviruses with H5 gene inserts have shown broad cross protection in chickens against challenge by diverse H5 HPAI viruses from Eurasia and North America (Swayne & Kapczynski, 2008). In 1995, Mexico implemented influenza A vaccine use for poultry as one tool in the HPAI control strategy, with eradication of HPAI strain by June 1995, but as H5N2 LPAI viruses continued to circulate, H5N2 vaccination was maintained (Villarreal, 2007). Within a few years, multiple lineages of antigenically variant H5N2 LPAI field viruses emerged that escaped from immunity induced by the original 1994 inactivated vaccine seed strain (Lee *et al.*, 2004). Similarly, emergent H5Nx HPAI Gs/GD lineage field viruses have arisen in China (the People's Rep. of), Indonesia, Egypt and various other Asian and Middle Eastern countries since 2005 that escaped from immunity induced by classical H5 inactivated LPAI vaccine seed strains and even rg generated H5 vaccine seed strains used in commercial vaccines (Grund *et al.*, 2011; Liu *et al.*, 2020; Swayne & Sims, 2020). Similarly, H9N2 LPAI field viruses resistant to inactivated vaccine seed strains have arisen in multiple countries in Asian and Middle East after prolonged usage of a single inactivated vaccine seed strain. It is not clear whether the emergence of these antigenic variants is related to use of vaccines or improper use of vaccines, but the emergence of resistance necessitated the change in vaccine seed strains to antigenically match the circulating field strains (Cattoli *et al.*, 2011; Lee *et al.*, 2016). China as the largest user of avian influenza vaccines has updated it's inactivated H5Nx (Gs/GD lineage) and H7N9 seed strains eight times and once, respectively, with the life span of a seed strain ranging from 3 to 7 years (Liu *et al.*, 2020; Swayne & Sims, 2020). Mexico has updated its H5N2 inactivated seed strains twice and its rFPV-H5 once over a 20-year period of H5 vaccine use (Swayne & Sims, 2020). Initially H9N2 inactivated vaccine usage in South Korea, was associated with decreased field virus diversity, as vaccinal immunity completely inhibited antigenically closely related field virus replication (Lee et al., 2016). However, over time, field

virus diversity increases as antigenic variants arise in the field and expand their populations. The live recombinant vectored vaccines have been updated less frequently, suggesting a broader immunity, requiring less frequent insert updates as compared inactivated vaccine seed strains.

All influenza A vaccination programmes should have an epidemiologically relevant surveillance programme that includes all relevant geographical regions and production sectors. The resulting isolates, along with viruses obtained from outbreaks, should be assessed for genetic and antigenic variation as part of an ongoing program for assessing vaccine effectiveness in the field. Initially, the viruses should be sequenced and analysed for critical amino acid changes within the five major antigenic epitopes of the HA. A representative subset of antigenic variants should be tested for cross-reactivity in a HI test using a panel of standard antisera produced against diverse influenza A viruses from the same HA subtype and the data analysed for quantitative changes by antigenic cartography (Fouchier & Smith, 2010). Based on this cartographic data, a few of the predominant circulating influenza A viruses and selected antigenic variants should be used in challenge efficacy studies (Swayne *et al.*, 2015). Vaccines that are not protective should be discontinued and replaced with vaccines containing updated inactivated vaccine seed strains or HA inserts within other vaccine platforms. Based on the timeline for emergence of antigenic variants for H5N1 viruses in China (People's Rep. of), vaccines should be assessed at a minimum every 2–3 years for efficacy against predominant circulating field viruses of the country or region. Alternatively, vaccine seed strains should be updated when a vaccine-escape mutant accounts for more than 30% of the relevant AIV subtype (Liu *et al.*, 2020). Based on this scientific information, the competent veterinary authority within the country should establish, in consultation with leading veterinary vaccine scientists and international organisations, naturally isolated or reverse genetics LPAI vaccine seed strains for conventional inactivated vaccines, and H5 and H7 haemagglutinin gene insert cassettes for recombinant vaccines. In some situations, more than one seed strain may be necessary to cover all production sectors within a country. Only high quality and potent vaccines should be approved for use in control programmes. Proper administration of high quality, potent vaccines is critical in inducing protective immunity in poultry populations.

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NB: There are WOAH Reference Laboratories for avian influenza (please consult the WOAH Web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3). Please contact the WOAH Reference Laboratories for any further information on diagnostic tests, reagents and vaccines for avian influenza

NB: FIRST ADOPTED IN 1989 AS AVIAN INFLUENZA (FOWL PLAGUE). MOST RECENT UPDATES ADOPTED IN 2021.

APPENDIX 3.3.4.1.

BIOSAFETY GUIDELINES FOR HANDLING HIGH PATHOGENICITY AVIAN INFLUENZA VIRUSES IN VETERINARY DIAGNOSTIC LABORATORIES

INTRODUCTION

The spread of high pathogenicity H5Nx avian influenza throughout Asia, Africa and Europe has led to an increase in the number of laboratories performing diagnostics for this pathogen. High pathogenicity avian influenza (HPAI) viruses, in general, are a serious threat to birds and mortality is often 100% in susceptible chickens. In addition, the agents can also pose a serious zoonotic threat, with approximately 60% mortality reported in humans infected with H5N1 HPAI virus. In recognition of the need for guidance on how to handle these viruses safely, the WOAH has established the following biocontainment guidelines for handling specimens that may contain HPAI virus. They are based on Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*, the World Health Organization[5], and Centers for Disease Control and Prevention[6].

BIOCONTAINMENT LEVELS

Samples for diagnostic testing for HPAI virus using the following techniques do not require high-level containment but should be carried out at an appropriate biosafety and containment level determined by risk analysis (see chapter 1.1.4.):

- Conventional and real-time reverse transcriptase polymerase chain reaction (RT-PCR)
- Antigen-capture assays
- Serology

Virus isolation and identification procedures for handling specimens that may contain high-titred replication-competent HPAI virus should as a minimum, include the following:

- Personnel protective equipment should be worn, including solid-front laboratory coats, gloves, safety glasses and respirators with greater than or equal to 95% efficiency.
- Specimens from potentially infected birds or animals should only be processed in type II or type III biological safety cabinets (BSC).
- Necropsies of birds should be performed in a Type II BSC while wearing respiratory protection, such as a N95 respirator, or in a Type III biological safety cabinet, or other primary containment devices with 95% efficient air filtration.
- Centrifugation should be performed in sealed centrifuge cups.
- Centrifugation rotors should be opened and unloaded in a BSC.

5 WHO laboratory biosafety guidelines for handling specimens suspected of containing avian influenza A virus, 12 January 2005.

6 Biosafety in Microbiological and Biomedical Laboratories, 5th edition. HHS Publication No. (CDC) 21-1112. https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF 1 December 2009.

- Work surfaces and equipment should be decontaminated after specimen processing.
- Contaminated materials should be decontaminated by autoclaving or disinfection before disposal or should be incinerated.

If chickens or other birds or mammals are inoculated with HPAI viruses, inoculation should be done in appropriate containment including:

- Inoculated chickens should be held in animal isolation cabinets or other primary containment devices, or non-isolation cages/floor pens in specially designed containment rooms
- Animal isolation cabinets should be in a separate facility that is equipped to handle the appropriate biocontainment for HPAI.
- The room should be under negative pressure to the outside and the animal isolation cabinets should be under negative pressure to the room.
- Animal isolation cabinets should have HEPA-filtered inlet and exhaust air.
- Biosafety cabinet or other primary containment devices should be available in the animal facility to perform post-mortem examinations and to collect specimens.

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Overview of how Canada prevents, prepares and responds to bird flu outbreaks

This is Exhibit "D" referred to in the affidavit of Katrina Jones sworn before me at White Rock this 30 day of January 20 25

On this page

Avian influenza (AI) is a contagious viral infection that can affect several species of poultry, such as chicken and turkey, as well as pet and wild birds. AI viruses can be classified into two categories-low pathogenic (LPAI) and high pathogenic (HPAI)-based on the severity of the illness caused in poultry. HPAI viruses typically cause severe illness and mortality, whereas LPAI viruses typically cause little or no clinical signs. Most AI viruses are low pathogenic; however, some subtypes are capable of becoming highly pathogenic. Historically, only the H5 and H7 LPAI virus subtypes are known to have the ability to become highly pathogenic and they are considered notifiable.

The Government of Canada attaches high priority to the threat of AI and is devoting significant resources to prevent the introduction and spread of AI in Canada. The Canadian Food Inspection Agency (CFIA) is at the forefront of that effort.

The CFIA, working with a number of Government of Canada partners, has

put in place a series of measures to limit the animal health risks-and associated economic repercussions of outbreaks-posed by AI. In the context of human health, these measures also reduce the potential risk that AI infection in birds will serve as the precursor to a human flu pandemic. International human and animal health authorities agree that efforts to protect human health are best directed at preventing, limiting and eradicating AI outbreaks in domestic poultry.

Prevention and early warning

There are a wide range of AI viruses continuously circulating within wild bird populations. The majority of these do not cause serious illness in animals or humans. The first lines of defence against an outbreak of AI in domestic poultry are prevention measures and early warning systems. The CFIA, in collaboration with other Government departments, has put in place safeguards to limit the introduction and spread of AI in Canada's domestic poultry populations.

Surveillance

The Canadian Government uses two different bird surveillance programs to detect AI viruses posing threats to domestic poultry at the earliest possible moment. The first program targets wild birds; the second one focuses on domestic flocks.

Wild bird surveillance

The CFIA, Environment Canada, the Public Health Agency of Canada and the Canadian Cooperative Wildlife Health Centre collaborate to conduct an annual survey of AI viruses in wild birds. The survey partners expect to find a variety of AI viruses, most of which commonly circulate in wild

birds with little or no impact on their health or the health of other animals. The survey includes sampling of live birds during the spring, summer and fall and continued year-round sampling of dead birds. The survey is intended to provide early detection of highly pathogenic AI in Canada and determine the presence and characteristics of the AI strains in North America's wild bird population.

Survey partners are particularly interested in AI viruses that are or have the potential to become highly pathogenic. These viruses, which include the H5 and H7 subtypes, can cause illness and death in poultry. The highly pathogenic H5N1 AI virus strain currently circulating in Asia, Africa and Europe has demonstrated the ability to affect poultry and wild birds, as well as humans and other mammalian species.

Survey results are reported as they are confirmed and are available at the Canadian Cooperative Wildlife Health Centre Website.

Commercial bird surveillance

The CFIA, in collaboration with industry, has designed a commercial bird surveillance program, called the Canadian Notifiable Avian Influenza Surveillance System (CanNAISS), to complement the wild bird survey. Samples are taken from live birds and tested in CFIA accredited labs. This survey helps us develop a better picture of AI viruses that might be circulating in Canadian poultry and can help to identify where breaches of on-farm biosecurity might have occurred and identify courses of corrective action. Additionally, abnormal patterns of flock productivity and mortality would be watched closely.

International bird surveillance

Through international cooperation and information sharing, Canada

continuously monitors AI developments around the world and adjusts import controls and disease response plans accordingly.

Biosecurity

AI virus can be transmitted directly from bird to bird through secretions and feces, and indirectly through human movement, contaminated feed, water and equipment. In light of the threat and risks associated with AI, increased attention has been drawn to the ongoing need to protect domestic poultry through the effective use of on-farm biosecurity measures. Biosecurity involves maintaining good hygiene practices and limiting exposure to external sources of contamination.

Commercial flocks

Most poultry and egg production industry associations already have biosecurity guidelines in place for their memberships to reference. The CFIA's role involves promoting best practices and providing technical advice across industry so that all producers are using the most effective measures possible and that these measures are being applied in a uniform fashion across the country.

Other flocks

The CFIA recognizes that not all poultry and egg production in Canada is done by large producers that are members of industry associations. There are smaller producers who maintain small flocks, or what may be called "backyard flocks." The CFIA, in collaboration with the provinces and territories, has implemented an awareness campaign for owners of these types of flocks to inform them of biosecurity best practices and encourage them to take the necessary steps to protect their flocks.

Import measures for live birds

These measures apply to countries which are recognized as being free of highly pathogenic AI in their domestic flocks. Canada continues to prohibit trade in poultry, poultry products and birds with any country until domestic poultry are proven to be free of highly pathogenic AI.

These measures are consistent with guidelines established by the World Organization for Animal Health (WOAH; founded as Office International des Épizooties (OIE)) and provide a foundation for safe trade while protecting animal and human health. Canada's import controls were developed in consultation with provincial governments, the Canadian poultry industry and Canada's principal poultry and bird trading partners: the United States and the European Union.

- Import measures for live birds to prevent the introduction of avian influenza in domestic birds

Emergency preparedness

While it is extremely important to have early warning systems and prevention measures in place to keep AI out of Canada, similar effort must be directed toward being prepared for the possibility of an outbreak. Since 2004, Canada has experienced one high pathogenic AI outbreak and testing has identified many different AI viruses, including H5 subtypes, which were determined to be low pathogenic. During these incidents, many valuable lessons are learned and experience is gained.

Emergency response team

The CFIA has a dedicated response team of experts that will be activated in the event of an AI outbreak. This group includes veterinarians,

executive management and field staff, will oversee the CFIA's response and coordinate actions with federal, provincial and municipal partners.

Development of detailed procedures for response

Preparedness requires that contingency plans be in place for every activity associated with an outbreak. Among the many detailed plans and procedures, there are plans for: humane and rapid destruction of infected flocks; minimizing the spread of virus; effective disposal of carcasses; movement restrictions on susceptible livestock and products; protecting the health and safety of staff deployed during an AI outbreak, protecting the health of farmers and producers during an AI outbreak, and capturing information in databases for epidemiological analysis of the outbreak.

Avian Influenza scenarios and exercises

The CFIA conducts a number of internal and external exercises to further enhance preparedness for a possible AI outbreak. Internally, the CFIA continues to enhance its ability to respond through ongoing emergency preparedness workshops and training events. Externally, the CFIA participates in exercises with industry as well as other government departments and levels of government to test response to AI in different parts of Canada.

Partnerships with other government departments, other levels of government and external bodies

The CFIA continues to work closely with other Government departments, other levels of government, the poultry and egg producing industries and the scientific and academic communities, all of which have a focus

on AI.

Partnerships with other federal government departments and agencies

At the Federal level, the CFIA's AI partners include, but are not limited to, Agriculture and Agri-Food Canada, the Department of Foreign Affairs and International Trade, Environment Canada, Health Canada, the Public Health Agency of Canada, Public Safety Canada and the Canada Border Services Agency.

The lead department or agency in the event of an AI outbreak is scenario dependent. If the scenario only involves animal health, then the CFIA will have the lead coordinating role in responding to the threat. If the scenario starts as an animal health issue, and then evolves into a human health issue, then the lead coordinating role would shift to the Public Health Agency of Canada. In the event that AI starts as a human health issue, the Public Health Agency of Canada would assume the lead role in coordinating the response.

The CFIA collaborates with these partner departments on AI and pandemic scenarios on an on-going basis.

Provinces and territories

The CFIA continues to communicate with its counterparts in the provinces and territories to ensure that information, policies, procedures, strategies, plans and communications products are shared and coordinated.

The CFIA, in collaboration with provincial governments, is continuously reviewing and updating the joint Foreign Animal Disease Emergency Support Agreements, which define the roles and responsibilities of each

partner in the case of a disease outbreak. These plans are based on four major disease control principles: rapid detection of newly infected livestock; halting the spread of the disease through movement controls and the rapid destruction of infected livestock; movement controls and surveillance on high risk livestock and proximal livestock; and preventing re-infection through the effective biocontainment of infective material (carcasses, manure and feed).

Industry and academia

The CFIA has solicited expertise from industry and the scientific and academic communities by striking an Avian Influenza Advisory Group. Representatives help to ensure that CFIA policies and action plans are sound. These consultations are ongoing and continue to provide valuable intelligence that helps to shape the CFIA's overall strategy to combat AI in Canada.

Partnerships with international bodies

The CFIA collaborates with leading international bodies such as the WOAH (World Organization for Animal Health), the Food and Agriculture Organization and the World Health Organization to share and distribute intelligence, and best practices with regard to combating Avian Influenza. The fight against AI is truly an international effort with many nations, including Canada, providing assistance to other areas of the world where resources may be limited and are needed to help contain the global spread of AI. This effort benefits all nations and serves the best interests of Canada.

The CFIA's National Centre for Foreign Animal Diseases in Winnipeg is recognized by the WOAH as an international reference laboratory for AI.

Additional capacity

It is understood that responding to an AI outbreak will require additional human resources, equipment and facilities. The CFIA determines how much "surge capacity" will be needed to address a specific threat and then develops unique contingency plans to add resources and capacity as needed. This is especially true for AI. Surge capacity planning with regard to AI focuses on the following areas:

Internal Staffing Reserve – ensuring the CFIA has enough staff, back-up staff and staff rotation for the duration of an AI outbreak.

External Staffing Reserve – ensuring that the CFIA has identified trained persons not currently on CFIA staff, but having relevant experience, so that they can be deployed during an AI outbreak, if required.

Equipment – ensuring that the CFIA can, at short notice, acquire and deploy the equipment required to address an outbreak of AI in Canada. This would include, but is not limited to, personal protective equipment for CFIA staff, vehicles, and depopulation equipment for the humane culling of infected animals.

Laboratories – Six provincial laboratories have been CFIA approved for AI sample testing. The CFIA maintains four labs of its own so that it can also conduct AI testing. CFIA lab staff can be mobilized to move closer to an AI outbreak anywhere in Canada.

Communications

The CFIA recognizes that communication is a key component in Canada's national effort to prevent, contain and eliminate AI outbreaks.

The CFIA maintains ongoing and frequent communications with federal

and provincial government partners, the animal health community, bird owners, industry, international disease control authorities and, most importantly, the Canadian public. Timely and transparent communication ensures that the most reliable and recent information is available to decision makers, stakeholders and Canadians. The CFIA recognizes that awareness and credible, science-based information are essential components of Canada's AI readiness and response capacity.

Response

In the event of an outbreak of AI in Canada, Canadians can be assured that the CFIA has action plans to guide effective and efficient response operations. These plans draw from previous experience in Canada and abroad, and the most current internationally accepted understanding of AI.

While specific response elements vary based on the virus and infected poultry species, the CFIA's actions generally include movement restriction, disease containment and surveillance components.

Disease containment

All infected flocks are humanely destroyed, and carcasses are disposed of in an environmentally acceptable fashion. Infected premises are thoroughly cleaned and disinfected before new birds can be introduced. Where highly pathogenic virus is present, flocks in the vicinity of infected premises and those from poultry operations that may have had contact with infected premises are also humanely destroyed and disposed of as a pre-emptive measure.

Surveillance, quarantine and segregation

Because each outbreak situation is unique, CFIA responses are flexible and may differ based on a variety of factors. For example, some disease response protocols are species specific. What follows, therefore, is the general approach to surveillance and segregation after an outbreak of AI in domestic poultry has been confirmed.

Quarantines restricting the movement of poultry and poultry products are placed on infected premises, poultry operations located in the vicinity of infected premises and other poultry operations that may have had contact with infected premises. Birds from quarantined premises are tested and monitored for evidence of AI infection

The CFIA may also ask domestic poultry producers to execute a segregation protocol. A segregation protocol seeks to minimize, if not eliminate, potential contact between wild birds and domestic or captive birds in the area after a case of HPAI has been confirmed.

- What to expect if your animals are infected
- Notifiable Avian Influenza Hazard Specific Plan

Vaccination against HPAI

Canada has historically maintained a stamping out policy for HPAI with the goal of achieving disease eradication in poultry and a return to disease-free status. However, the scale and duration of the recent outbreak, along with international movements towards exploring the use of vaccination as an additional tool to fight against HPAI, has prompted Canada to take action. In response, a task force was formed in June 2023 to study the challenges and opportunities for the development and implementation of an HPAI vaccination program.

- Highly Pathogenic Avian Influenza Vaccination Task Force

Date modified:

2023-11-20

Highly Pathogenic Avian Influenza Vaccination Task Force

Related links

- Avian influenza (bird flu)
- Latest bird flu situation in Canada
- Detections of highly pathogenic avian influenza in Canada
- Overview of how Canada prevents, prepares and responds to bird flu outbreaks
- Fact Sheet – Avian Influenza

The Highly Pathogenic Avian Influenza (HPAI) Vaccination Task Force is dedicated to studying the challenges and opportunities for the development and implementation of an HPAI vaccination program.

This task force serves as a forum for discussion and information sharing that brings together insights from veterinarians, experts from academia, industry representatives and government representatives on issues relating to the potential use of vaccination against HPAI in Canada.

Background

The recent outbreak of H5N1 HPAI has resulted in the deaths of hundreds of millions of domestic and wild birds throughout the globe. HPAI has occurred in areas of the world where it had never occurred previously, such as countries in Central and South America. In Canada, millions of birds have been impacted since December 2021.

Canada has historically maintained a stamping out policy for HPAI with the goal of achieving disease eradication in poultry and a return to disease-free status. However, the scale and duration of this outbreak, along with international movements towards exploring the use of vaccination as an additional tool to fight against HPAI, has prompted Canada to take action. 70

In response, this task force was formed in June 2023 building on what has been done to date to bring government, experts and stakeholders together for discussion and consensus building regarding the potential use of vaccination against HPAI in Canada. The task force also informs the Canadian Food Inspection Agency's (CFIA) decision making process regarding the potential implementation of a vaccination program.

Topics of discussion

The task force is exploring whether Canada would benefit from a vaccination program. Topics of discussion include and are not limited to:

- availability of effective vaccines
    - what vaccines are available, for which species
- implementation considerations
    - logistics
    - roles and responsibilities of government, industry and veterinarians in a roll-out
- approaches for surveillance
    - requirements for differentiating infected from vaccinated animals (DIVA) methodology
    - how to meet surveillance requirements set by key trading partners

- trade implications
    - assessing potential trade implications that could result from vaccination, particularly for Canada's export markets
- identifying cost and benefits
    - cost of vaccines per dose
    - administration of vaccine
    - surveillance
    - assessment of economic costs and benefits to industry and government
    - cost and responsibility sharing
- knowledge exchange and identifying data gaps
    - international experiences/lessons learned
    - results of field trials
    - identifying Canadian-specific research needs
- any other considerations that may be relevant to the work of the task force
    - identification of any challenges or barriers, opportunities, and lessons learned

Looking ahead

- The HPAI Vaccination Task Force may inform:
    - policies or strategies developed by the CFIA that would outline conditions for vaccination in Canada, including which species to vaccinate in which region(s) in the event of HPAI vaccination
    - design and implementation of a potential vaccination program

Members

The task force is co-chaired by the CFIA and an industry representative.

Co-chairs

- Dr. Mary Jane Ireland, Chief Veterinary Officer for Canada, Canadian Food Inspection Agency
- Phil Boyd, Executive Director, Turkey Farmers of Canada

Members include industry representatives, veterinarians, academia experts and government representatives:

- Agriculture and Agri-food Canada
- Animal Health Canada
- Canada's Accredited Zoos and Aquariums
- Canadian Association of Poultry Veterinarians
- Canadian Food Inspection Agency
- Canadian Hatching Egg Producers
- Canadian Poultry and Egg Processors Council
- Canadian Poultry Genetics Exporters Association
- Canadian Veterinary Medical Association
- Chicken Farmers of Canada
- Egg Farmers of Canada
- Environment and Climate Change Canada
- Équipe québécoise de contrôle des maladies avicoles (EQCMA)
- Provinces and territories / Council of Chief Veterinary Officers
- Public Health Agency of Canada
- Representative of duck veterinarians
- Representatives of genetics / breeding sector
- Turkey Farmers of Canada
- University of Guelph

Additional information

- Animal Health Offices

Date modified:
2024-06-11

2.7 Immunity

2.7.1 Active

Infection with, or exposure to, AI viruses, as well as immunization with vaccines, stimulates an antibody response at both the systemic and the mucosal levels. A systemic Immunoglobin M response by five days post-infection is followed shortly by an Immunoglobin G response. The intensity of the antibody response varies with bird species, in the following order (from most intense to least):

1. chickens;
2. pheasants;
3. turkeys;
4. quail; and
5. ducks.

Antibodies against the surface proteins are neutralizing and protective. Protection has been primarily associated with antibodies directed to the HA protein; however, the presence of either HA or NA antibodies, or both, prevents clinical signs and death following challenge with HPAI viruses having homologous HA or NA subtypes. The level of protection against mucosal infection and subsequent shedding of the challenge virus may depend on the degree of sequence similarity in the HA of vaccine and challenge virus. The duration of protection is variable and depends on many factors, but in laying hens, protection against clinical signs and death has been demonstrated to be at least 30 weeks following a single immunization.

Immune response against internal proteins has not been shown to prevent clinical signs or death, but may shorten the period of virus replication and consequently reduce the shedding.

2.7.2 Passive

Studies on protection by maternal antibodies from homologous HA or NA have not been reported, but based on the available information about other viral avian diseases, protection against clinical signs and death from a homologous AI viral challenge is probable for the first two weeks after hatching. For surveillance purposes, the WOAH suggests that maternal antibodies derived from a vaccinated parent flock are usually found in the yolk and can persist in progeny for up to four weeks.

2.7.3 Vaccination

The modernized approach of the WOAH and the scientific community regarding AI vaccination makes vaccine use more acceptable. Vaccination has been used in various poultry species, and its effectiveness in preventing clinical signs and mortality is well documented. Developed countries should aim for eradication without the use of vaccines when facing a NAI outbreak. As part of preparedness for a disease outbreak, countries should identify available sources of NAI vaccines in advance.

This is Exhibit "F" referred to in the
affidavit of Katrina Jones
sworn before me at Waterloo
this 30 day of January 20 25

Open and Transparent Agency Policy

This is Exhibit C referred to in the affidavit of Katrina Jones sworn before me at White Rock this 30 day of January 20 25

On this page

1. Background

1.1 The CFIA's vision is to excel as a science-based regulator, trusted and respected by Canadians and the international community. To this end, preserving public confidence in the CFIA's (the Agency's) decisions and activities is key to protect its credibility and reputation. 76

To maintain public trust, the CFIA is committed to providing Canadians with information about its publicly-funded regulatory and scientific activities. Canadians are entitled to this information and it can help them to make informed decisions for themselves, their families, and businesses.

Transparency and openness are key values underpinning the CFIA's activities. As part of its ongoing evolution toward becoming a more responsive and accountable organization, the CFIA initially began to release more information about its decisions and activities in 2011 through its Transparency Agenda.

1.2 This policy represents a refresh to the CFIA's approach to openness and transparency, formalizes Agency practice and provides CFIA direction for implementing the next stage of its Transparency Agenda. This means the Agency will:

1.2.1 build on and expand existing transparency practices realized since the Transparency Agenda was first implemented

1.2.2 undertake new practices in order to keep pace with evolving public expectations influenced by transparency initiatives of other regulators both in Canada and around the world

1.2.3 proactively identify opportunities to make information– such as reports, documents, decisions and data – publicly available throughout the lifecycle of the Agency's programs and activities

1.2.4 embed openness and transparency into our programs, decisions and activities whenever possible by building them in from their inception as part of attaining the Agency's vision of becoming open by design

1.3 These practices and the CFIA's commitment to disclosing relevant information to its stakeholders go hand in hand with the direction taken by the Government of Canada's Open Government and Open Science initiatives.

1.4 This policy provides direction to CFIA employees in line with its obligations to comply with Treasury Board Secretariat of Canada (TBS) requirements to maximize release of data and information of business value to stakeholders. It should be read alongside the TBS Directive on Open Government, which provides mandatory Government of Canada requirements to become open by default, and in turn influences the CFIA's goal of becoming open by design.

2. Policy statement

The CFIA is open by design and releases relevant, accurate, and timely information to stakeholders about its regulatory and scientific activities, decisions, programs and services.

3. Objectives

The objectives of this policy are to:

3.1. preserve trust in Canada's regulatory system for food, plants and animals, by demonstrating visible and public accountability for delivery of the CFIA's regulatory programs and services

3.2. better inform Canadians about the CFIA's mandate to protect Canada's food, plants and animals, and provide information that will enhance their ability to make informed decisions for themselves, their families and their businesses

3.3. contribute to a fair, competitive business environment for regulated parties by providing tools to clarify industry's role in meeting regulatory requirements and information about compliance outcomes

3.4. provide consistent direction to all CFIA employees and clarify the important role they will play in supporting the Agency to deliver on its commitment to be open by design

4. Scope

This policy applies to the following:

4.1 All CFIA employees as well as temporary, and term staff

4.2 Contractors and students engaged by the Agency, subject to the terms and conditions of their contract

4.3 All CFIA information, except that which will not be disclosed in line with section 8 of this policy and other requirements of the *Access to Information Act* and *Privacy Act*

5. Authorities

This policy supports CFIA compliance with mandatory Government of Canada requirements issued by TBS under section 7 of the *Financial Administration Act*.

Relevant legislation relating to release of Government information is as

follows:

- *Access to Information Act*
- *Official Languages Act*
- *Privacy Act*

6. Guiding principles

The CFIA's Transparency Agenda is:

Open by Design

- Openness and transparency are integrated throughout the entire lifecycle of CFIA programs, policies, services and enabling technologies. From inception, consideration is given to how information generated will be publically released

User-Centric

- Relevant, accurate, and timely information is shared proactively with stakeholders, without waiting for an access to information request
- Context is provided so that both potential possibilities and limitations of use are clearly communicated

Inclusive

- Stakeholders and end-users are consulted and engaged as required to ensure openness and transparency initiatives are service-oriented and meet their intended objectives
- The Agency maintains, and is seen as maintaining, its regulatory independence

Diligent

- Consequences and impacts (both positive and negative) of providing information are fully considered and balanced prior to release
- Private and confidential information belonging to individuals and third parties is appropriately protected

Agile

- The CFIA's Transparency Agenda evolves and is responsive both to shifts in public and government expectations and changes in its operating environment as part of ensuring it is sustainably implemented

7. Requirements

7.1 CFIA information must be released in accordance with this policy and any applicable CFIA release procedures

7.2 Information must be released in a timely manner that allows users to derive maximum benefit from them for decision-making purposes

7.3 The CFIA shall prioritize release of information that:

7.3.1 is of high public interest

7.3.2 relates to Agency and Government of Canada priorities

7.3.3 contributes to informed decision making by Canadians about products they consume and/or use

7.3.4 promotes compliance by industry and the public with CFIA regulations

7.3.5 is frequently requested through channels including Access to Information requests, the Government of Canada's Open Government

Portal, informal requests received by the CFIA, media requests, and regular communications between the Agency and its stakeholders during the course of program delivery and engagement activities

7.4 All CFIA staff must continuously consider whether and how key information relating to the Agency's programs and services can be publicly released as part of their design, re-design, and approval

7.5 Open by design features that support transparency must be integrated into new information technology (IT) tools at their inception, built into older systems during upgrades, and be capable of releasing information to the public upon implementation

7.6 Agency information intended for the public must be created using plain language, contextualized, and made understandable by the broadest audience possible, maintaining scientific and technical rigour as appropriate

7.7 The CFIA organizes information that is released logically, visibly, in a downloadable format and accessible location that facilitates access by stakeholders

7.8 To facilitate release, information generated by CFIA programs and services must be created, stored and managed in compliance with approved information management (IM) and data management (DM) standards

7.9 Information posted to the CFIA website as a dataset must also be formatted in a machine-readable format and made available on the Government of Canada's Open Data Portal as an open dataset

7.10 Outcomes and outputs of initiatives that are part of the CFIA's Transparency Agenda, once completed and made publicly available:

7.10.1 shall, in consultation with and at the discretion of the Communications and Public Affairs Branch, be accompanied by any appropriate communications to inform stakeholders of their availability, including details such as where and how they may be accessed

7.10.2 if accompanied by formal communications, must be framed as part of the broader narrative about the Agency's openness and transparency objectives, through a business-line lens

7.11 Information that is confirmed to contain personal and/or confidential information must undergo further analysis to determine if it can be re-formatted or redacted to enable release

7.12 Decisions made not to release information that supports the objectives of this policy must be documented and include a rationale that references key considerations based on policies, standards and legislation as appropriate

7.13 Documentation noted in section 7.12 must be formatted and stored in a way that it can be made available upon request as part of facilitating reviews of the Agency's approach to openness and transparency, including those that may be initiated under section 11.1 of this policy

8. Exceptions

The CFIA may not disclose information that contains personal and/or confidential information. This includes and is not limited to information that:

8.1 is personal in nature or could lead to the identification of an individual or other people

8.2 belongs to third parties and is considered confidential business information

8.3 would harm the CFIA's ability to enforce its legislation, such as information about specific investigative techniques and investigations in progress

8.4 is scientific or technical information obtained through research and is awaiting publication, and if disclosed could reasonably be expected to deprive the employee of priority of publication

8.5 contains advice or recommendations developed for Ministers and/or Cabinet, and that are protected by the convention of Cabinet confidence

8.6 may harm relations or negotiations with any international, indigenous, provincial, territorial or municipal government

8.7 may threaten the safety of a person or present a risk to the security of any property or system

9. Roles and responsibilities

9.1 The CFIA President:

- Provides leadership on development of a culture of open by design at all levels throughout the Agency
- Incorporates commitments related to openness, transparency, and open by design into performance agreements of the Agency's senior management cadre, or other methods and instruments as appropriate as part of fostering this culture

9.2 Vice Presidents and special officers:

- Promote a culture of open by design within their branches and

organizations by clearly communicating expectations to employees and expected outcomes 84

- Verify that the requirements of this policy are met in Branch initiatives, programs and activities
- Apply a One Agency lens, ensuring that requirements of this policy are integrated into initiatives considered by CFIA Governance
- Ensure that the requirement to build in open by design to CFIA programs, services and initiatives is accounted for when developing budgets and allocating resources

9.3 The Digital Services Branch:

- Defines standards and guidelines for building openness and transparency into the CFIA's information management (IM) and information technology (IT) solutions to facilitate release of information to the public
- Includes or incorporates requirements for openness and transparency in specifications for IT system solutions
- Collaborates with CFIA groups to design and build system solutions that take into account these requirements for openness and transparency throughout the information lifecycle

9.4 Executives and Program Managers:

- Apply and promote the principles and requirements in this policy to their work units and identify eligible information for release
- Responsible for developing and documenting decisions within their group to comply with section 7.12 of this policy
- Ensure that human and financial resources to support openness and transparency elements of work undertaken by their units are considered and integrated into workplans and budgets

9.5 All CFIA employees:

- Apply the guiding principles and requirements of this policy to their day-to-day work
- Continuously seek to identify information and/or changes to business processes that will help the Agency attain its vision of being open by design

9.6 Legal Services and Access to Information and Privacy (ATIP – Integrity and Redress Secretariat):

- Assess the implications of proactively posting information under existing laws and advise that Agency practices comply with legal requirements
- Provide advice and options as to how information that cannot be released due to valid exceptions including confidentiality, privacy and security implications, can be redacted, edited or reshaped into a compliant format

10. References

10.1 Related policies and direction

Government of Canada

- Policy on Communications and Federal Identity
- Cabinet Directive on Regulation

Treasury Board of Canada Secretariat

- Policy on Access to Information
- Policy on Results
- Directive on Open Government
- Directive on Recordkeeping

- Canada's 2018-2020 National Action Plan on Open Government

Canadian Food Inspection Agency

- Stakeholder Engagement Framework
- Compliance Promotion Communications Framework
- Science Branch Scientific Publication Policy
- Consultation Policy and Framework
- CFIA Recorded Information Management Policy
- Information Governance and Information Management Roles and Responsibilities Directive
- Open Government Implementation Plan (OGIP)

10.2 Related Resources for CFIA employees

- Open & Transparent Agency Policy – Guidance Document for staff (in development)
- CFIA Open Government Portal Dataset Publishing Procedure

11. Monitoring and Reporting

11.1 The Program Policy Integration Division (PPID) in Policy and Programs Branch is responsible for maintaining this policy, and reviewing it every five years, or earlier if changes are made to any of the following:

11.1.1 The 2014 TBS Directive on Open Government

11.1.2 Changes to the *Access to Information* and/or *Privacy Act*, including any changes in common law as interpreted by the courts

11.1.3 Changes to CFIA legislation and regulations

11.2 Results of reviews conducted under section 11.1 will be reported to

the Agency's Information Governance Committee (IGC), chaired by the CIO/IMSO delegate, the CFIA's Chief Data and Risk Officer in the Digital Services Branch (DSB), and to Program and Policy Management Committee (PPMC) as required for information

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12. Inquiries

Send questions or comments about this policy:

- **By e-mail:**
  transparency.transparence@inspection.gc.ca
- **By mail:**
  Director
  Program Policy Integration Division
  Policy Branch
  Canadian Food Inspection Agency
  1400 Merivale Road
  Ottawa, ON, Canada K1A 0Y9

13. Effective Date

This policy replaces the CFIA's 2013 policy on Transparency in Regulatory Decision Making and comes into effect May 1, 2019.

Annex 1: Definitions

Confidential Business Information (CBI):
As defined in section 20 of the *Access to Information Act.*

Data:
Digital structured information residing in fixed fields, such as relational

databases or spreadsheets, raw facts, and statistics with no specific context.

Information:

Is comprised of both structured (data) and unstructured (records) resources. Records are electronic and physical unstructured information such as documents, web pages, media and print. Data are electronic, structured information in fixed fields such as relational databases.

Open by default:

An organizational culture that favours disclosure over non-disclosure - A broad principle that favours releasing government information of value to Canadians, with information being withheld only for necessary privacy, confidentiality and security reasons.

Open by design:

Refers to strategies that are used to ensure that openness and transparency considerations are deliberately and thoughtfully hard-wired into the design phase of all CFIA programs and services, and integrated when improvements are made to existing ones.

Open government:

A governing culture that holds that the public has the right to access the documents and proceedings of government to allow for greater openness, accountability, and engagement.

Open science:

A commitment related to Open Government that seeks to maximize access to federally funded scientific research to encourage greater collaboration and engagement with the scientific community, the private sector, and the public.

Openness:

Receptive to free exchange of information, communications, change and new ideas as part of seeking excellence and continual improvement in

design and delivery of programs and services.

Personal information:

As defined in section 3 of the *Privacy Act*

Plain language:

Writing that is clear, concise, well-organized and formatted in a way that maximizes the chance that the reader will quickly find the information they need, understand it the first time they read it, and then be able to take any appropriate action based on that understanding.

Record:

Digital and physical unstructured information, such as e-mail messages, Word documents, web pages, media and print – data that has been interpreted and organized, adding context and meaning.

Release:

Make publicly available online in a downloadable format.

Relevant:

Addresses and is responsive to a demonstrable need, and/or communicates information about a program, policy or other entity that is a government priority or a federal responsibility.

Stakeholder:

An entity either internal or external to the CFIA that has an interest in the Agency's programs and services, or their related activities, resources or deliverables, such as regulated parties, individual companies and representative industry associations, academia, Canadians, consumers, and other levels of government.

Timely:

Information is made available within a timeframe that will maximize its usefulness to users.

Transparency:

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Proactively providing relevant, accurate and timely information to the public to demonstrate accountability for delivery of programs and services, as part of supporting the right of Canadians to government information.

Date modified:

2023-12-14

This is Exhibit "11a" referred to in the
affidavit of Katrina Jones
sworn before me at Whitehorse
this 30 day of January 2025

CFIA's Policy for Providing Guidance on Regulatory Requirements

This policy outlines the commitments, practices, and tools applied by the Canadian Food Inspection Agency (CFIA) when providing Canadians and businesses with information and guidance on meeting regulatory obligations. It also identifies the conditions under which the CFIA will provide written responses to enquiries.

Agency context

The CFIA's core responsibility is to protect Canadians by safeguarding Canada's food system and the plant and animal resources on which we depend, and supporting the Canadian economy through the trade of Canadian goods.

The CFIA is committed to being fair and consistent in the application of regulations. The CFIA will, to the extent possible, endeavour to provide information to assist regulated parties and stakeholders in understanding their regulatory obligations. The following describes the CFIA's commitments in the areas of building awareness of regulatory requirements, responding to enquiries, service and stakeholder engagement.

Plain language commitment

- The CFIA (Canadian Food Inspection Agency) commits to have its regulations and guidance documents drafted in as plain language [1] as possible, limiting the use of specialized or technical language to those instances where it is necessary
- The CFIA (Canadian Food Inspection Agency) is committed to the publication of Frequently Asked Questions (FAQs) for all new or amended regulations that have business impacts. The CFIA (Canadian Food Inspection Agency) has posted FAQs (Frequently Asked Questions) for its most accessed regulations [2] to its website
- The CFIA (Canadian Food Inspection Agency) is committed to publishing FAQs (Frequently Asked Questions) for the areas of improvement identified through stakeholder check-ins, as well as for recurring questions

Building an awareness of regulatory requirements

- The CFIA (Canadian Food Inspection Agency) is committed to communicating regularly with Canadians and its stakeholders to promote awareness and understanding of regulatory compliance requirements, through compliance promotion activities and use of

online consultation/surveys, webinars and town halls, to facilitate the continual development of regulations and guidance tools

- The CFIA (Canadian Food Inspection Agency) is committed to developing products, such as guidance material, that are adapted to the needs of each regulated sector
- The CFIA (Canadian Food Inspection Agency) is committed to improving the accessibility of information regarding regulations and policies for regulated parties by making greater use of centralized services where there is a benefit to regulated parties. For example, the CFIA (Canadian Food Inspection Agency) is making regulatory guidance documents more accessible to stakeholders through its online Guidance Document Repository, a single repository for all guidance documents

Responding to enquiries

- The CFIA (Canadian Food Inspection Agency) is committed to responding to inquiries by stakeholders and Canadians in, to the extent possible, plain language, and in a clear, consistent and professional manner, in the official language of inquirer's choice in accordance with the requirements found in the *Official Languages Act* (OLA), and the Agency's Policy on Official Languages, in the form that the enquiries are made, either orally or written, or as appropriate
- The CFIA (Canadian Food Inspection Agency) commits to responding to enquiries in a timely manner. The CFIA (Canadian Food Inspection Agency) strives to acknowledge receipt of enquiries within five business days. The CFIA (Canadian Food Inspection Agency) does not provide legal advice to third parties about how specific regulations may apply to particular circumstances
- FAQs (Frequently Asked Questions) addressing recurring enquiries are posted to the CFIA (Canadian Food Inspection Agency)'s website

Service

Commitment to professional service

- The CFIA (Canadian Food Inspection Agency) is committed to provide timely, professional, courteous, impartial and respectful service, in both official languages as appropriate. A modern, digital service strategy is a key priority at the CFIA (Canadian Food Inspection Agency)
- As a science-based regulator, the CFIA (Canadian Food Inspection Agency) commits to service excellence and ensuring industry understands its role, responsibilities and accountabilities through robust compliance promotion activities, and standardized,

modern and user-friendly services. The CFIA (Canadian Food Inspection Agency) strives for continuous improvement in its processes and practices

Accountability

- The CFIA (Canadian Food Inspection Agency) provides services that are consistent with its regulatory obligations. The CFIA (Canadian Food Inspection Agency) has published a Statement of Rights and Service for Producers, Consumers and Other Stakeholders , as well as six accompanying guides to inspection to offer stakeholders and CFIA staff a clear, plain language explanation of the CFIA (Canadian Food Inspection Agency)'s commitment to:
  - transparent decision making
  - accessible and timely information
  - fair, respectful and unbiased interactions with stakeholders; and
  - responsiveness and continuous improvement
- To support continuous improvement to regulatory program delivery, transparency and predictability, the Complaints and Appeals Office provides an avenue for stakeholders to register complaints and appeals related to quality of service, administrative errors and regulatory decisions

Staff training

- The CFIA (Canadian Food Inspection Agency) continues to support employees by providing them with the necessary training to deliver high quality, professional services and to provide accurate, consistent and up-to-date information on regulatory requirements

Stakeholder engagement

As a regulator, the CFIA (Canadian Food Inspection Agency) engages with stakeholders, including the following groups:

- Regulated parties: individual companies and, in some cases, academia, including Official Language Minority post-secondary institutions, and government institutions, including Industry Value Chain Round Tables and Industry led Advisory committees
- Non-governmental organizations: representative industry associations and groups, and other non-governmental organizations
- Indigenous and other cultural groups
- Canadians: including consumers and consumer associations and groups, and other non-regulated parties such as micro and small businesses

- Other federal government departments and other levels of government: provincial, territorial and municipal governments, as well as established Federal/Provincial/Territorial mechanisms
- International stakeholders: foreign governments, international organizations
- Official Languages Minority Community (OLMC) organizations and industry groups.

Commitment to stakeholder engagement

- The CFIA (Canadian Food Inspection Agency) is committed to transparency, through the ongoing application of the Agency's Transparency in Regulatory Decision Making Policy
- The CFIA (Canadian Food Inspection Agency) is committed to engaging and consulting with Canadians and other stakeholders, as appropriate, to understand their perspectives on significant regulatory, policy and program issues that impact them. To develop effective policies and strategies, the CFIA (Canadian Food Inspection Agency) actively seeks out and values the perspectives of stakeholders who are affected by its decisions [3]

Stakeholder engagement practices

- The CFIA (Canadian Food Inspection Agency) engages regularly with its stakeholders through a variety of mechanisms in the regulatory development process. The CFIA (Canadian Food Inspection Agency)'s Consultation Policy and Framework aims to improve transparency and reduce duplication of efforts through an integrated, coordinated and consistent consultation and engagement process. As example, to foster ongoing dialogue, the CFIA (Canadian Food Inspection Agency) further leverages stakeholder involvement through:
    - Industry Value Chain Round Tables
    - Industry led Advisory committees
    - Federal/Provincial/Territorial mechanisms
- The CFIA (Canadian Food Inspection Agency) take proactive measures to consult with Official Languages Minority Community organizations, where appropriate, and in keeping with its obligations under Part VII (7) of the *Official Languages Act* related to the advancement of English and French
- Consultation opportunities can be found on CFIA (Canadian Food Inspection Agency)'s website and the Consulting with Canadians page on the website of the Government of Canada
- Stakeholders are also made aware of consultation opportunities through posting of the Forward Regulatory Plan on CFIA (Canadian Food Inspection Agency)'s website

- In addition to consultations, issues and concerns raised by regulated and non-regulated parties during ongoing contact are considered in the development of related materials and other additional outreach activities

Inquiries

For interpretation, clarification or inquiries regarding this policy please contact:

Director,
Regulatory, Legislative and Economic Affairs Division
Policy and Regulatory Affairs Directorate
Policy and Programs Branch
cfia.legislation-legislation.acia@inspection.gc.ca

Footnotes

1 Communications Policy of the Government of Canada

2 "Most accessed regulations" was determined by the number of times a regulation was accessed on Department of Justice's website in FY (fiscal year) 2013/2014.

3 Canadian Food Inspection Agency's Statement of Values

Date of last revision of this policy

2019-03-14

For more information

All of the government's Acts and Regulations can be found on the Justice Laws website.

Consult the following for links to the **Cabinet Directive on Regulation** and supporting policies and guidance, and for information on government-wide regulatory initiatives implemented by departments and agencies across the Government of Canada:

- Federal regulatory management
- Learn more about regulatory cooperation

To learn about upcoming or ongoing consultations on proposed federal regulations, visit:

- Consulting with Canadians
- *Canada Gazette*

Date modified:
2019-04-03

Field-ready lateral flow test for avian influenza

From: Innovation, Science and Economic Development Canada

The Canadian Food Inspection Agency (CFIA) is seeking an easy-to-use, rapid, and affordable lateral flow test to detect Avian Influenza (AI) and at the same time, H5 and H7 subtypes, with the same sensitivity and specificity as current molecular diagnostic tests used in the CFIA laboratory.

Challenge sponsor:
Canadian Food Inspection Agency (CFIA)

Funding mechanism:
Grant

Opening date:
August 30, 2023

Closing date:
October 11, 2023, 14:00 Eastern Time

This is Exhibit "J" referred to in the affidavit of Katrina Jones sworn before me at White Rock this 30 day of January 2025

Log in to view your submissions

Prospective applicants should refer to the Innovative Solutions Canada Grant Instructions and Procedures.

▼ Challenge

Problem statement

Avian influenza (AI), commonly known as "bird flu", is a contagious viral disease that primarily affects birds, particularly poultry. AI viruses are classified into 2 categories: low pathogenicity (LPAI) and high pathogenicity (HPAI) viruses, based on the severity of the illness caused in gallinaceous poultry. Wild birds, such as waterfowl and shorebirds, are natural reservoirs for influenza viruses, and the migration of these birds can lead to the spread of these viruses

across territorial boundaries. In humans, transmission can occur through close contact with infected birds or heavily-contaminated environments. In addition, on rare occasions, the HPAI virus can cause illness and sometimes death in humans.

The Canadian Food Inspection Agency (CFIA) has been actively addressing the current HPAI outbreak that began spreading within Canada starting in late 2021. HPAI infections in chickens and other flocks pose a threat to the poultry industry, leading to significant economic losses as infected birds may need to be culled to prevent the further spread of the disease. In 2022, exports of chicken and turkey were down by 32.3% and 8.7%, respectively compared to 2021 [1].

AI is currently diagnosed in CFIA laboratories by molecular methods that detect the genome of the virus using different types of real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays. Although these diagnostic tests are highly sensitive and specific, they are not easy to perform in the field causing a delay in generating results to farm staff in the field in a timely and accessible manner. This creates challenges when trying to rapidly and efficiently respond to an outbreak.

It has been demonstrated that lateral flow assays are able to detect different pathogens, including AI; however, the current lateral flow technology is not an appropriate substitute for rRT-PCR tests. The lack of sensitivity and specificity of current lateral flow tests are critical factors preventing these tests from being deployed and used in the field for diagnostic purposes.

This challenge aims to develop an easy-to-use, rapid, and affordable lateral flow test to detect AI and at the same time, H5 and H7 subtypes, with the same sensitivity and specificity as current molecular diagnostic tests used in the CFIA laboratory. The development of this technology would equip farmers, veterinarians, and CFIA inspectors with an easy-to-use tool to test animals for AI, which in turn could support the early detection of disease to help control and stop the spread of the disease.

Desired outcomes and considerations

Essential (mandatory) outcomes

The proposed solution must:

1. Be a lateral flow device that detects all avian influenza viruses in a oropharyngeal and cloacal swabs using the conserved regions of influenza A (e.g. nucleoprotein or matrix segments)
2. Be able to identify subtype H5 and H7 avian influenza viruses
3. Have a sensitivity approaching a cycle threshold range of 32 to 35, matching molecular assays (rRT-PCR assays) currently used for diagnostics

4. Have reliability and repeatability with high specificity similar to rRT-PCR assays
5. Provide a reliable qualitative signal (minimal probability of false positives and false negatives of > 2% indicating virus presence when concentrations are below a cycle threshold of 32per sample)
6. Have robust capacity to operate and produce results under varied temperature conditions (i.e. 5-35 degrees Celsius)
7. Be of a size and weight that makes the unit portable by a single individual for field use
8. Must be user-friendly (easy to use without any technical training or be used by non-experts)
9. Must be able to generate results in 30 minutes or less from the time of nasal swab acquisition
10. Be more cost-effective then molecular testing performed in a reference laboratory (not exceeding $20/test)

Additional outcomes

The proposed solution should:

1. Be suitable to identify the presence of influenza A virus antigen in animal tissue homogenate (e.g. gastrointestinal tract or nervous tissue)
2. Provide a reliable secondary or alternative qualitative signal when virus presence is below a cycle threshold of 32 in a sample
3. Provide a reliable signal intensity which increases in correlation with increased virus concentrations within the sample

Background and context

Avian influenza (AI), commonly known as "bird flu", is a contagious viral infection that can affect several species of commercial poultry species, as well as domestic and wild birds. AI viruses can be classified into 2 categories: low pathogenicity (LPAI) and high pathogenicity (HPAI) viruses, based on the severity of the illness caused in gallinaceous poultry species. Most AI viruses are low pathogenicity—these typically cause little or no signs of illness in infected birds. However, high pathogenicity AI viruses can cause severe illness and mortality in birds.

AI viruses are divided by subtypes based on 2 glycoproteins found in the surface of the virus: hemagglutinin, or "H" protein, and neuraminidase, or "N" protein. There are 16 H types and 9 N subtypes, leading to a total of 144 combination of possible virus subtypes. The H5 and H7 virus subtypes are of particular concern, given their ability to mutate from low to high pathogenicity after they infect gallinaceous poultry species. These 2 H-subtype viruses have been known to cause serious disease or mortality in domestic poultry, yet low pathogenic H5 and H7 viruses are quite common in wild waterfowl.

Avian influenza viruses have evolved into two phylogenetically different lineages (North

American and Eurasian) owing to natural geographic barriers, and separate distribution and migration of waterfowl. In a rare situation, these viruses can move across these barriers along continental margins in the Pacific and Atlantic parts of Canada where some of the migratory flyways overlap. As a result, exchange of gene segments from viruses belonging to both lineages or dispersal of complete genetically diverse strains takes place. For example, the H5N1 strain that has been reported in various parts of Europe is distinctly different from the Asian strain. In Canada, HPAI and low pathogenicity H5 and H7 avian influenza viruses are a reportable disease under the Health *of Animals Act* [2]. All cases must be reported to the CFIA.

HPAI (e.g., H5N1) outbreaks pose a significant risk on Canada's poultry industry and can have widespread consequences including high rates of poultry mortality, culling of birds to control the spread of disease, and impacts on producers' ability to export their animals. In addition, some HPAI virus strains like the A/Goose/Guangdong/1/1996 (GsGD linage) H5NX viruses can have effects on wildlife – mortality has been observed in a broad range of species ranging from wild birds, with sporadic spill over to domestic and wild carnivore mammals such as dogs and cats—to skunks, foxes, and marine mammals (dolphins and seals).

The current gold-standard for infectious disease diagnostics, including AI, requires laboratory confirmation of the disease. The National Centre for Foreign Animal Disease (NCFAD) in Winnipeg is a World Organisation for Animal Health (WOAH) reference laboratory for AI. This is where AI detections in Canada are confirmed by molecular methods (rRT-PCR) followed by virus isolation and genome sequencing. As a first step, a rRT-PCR assay is used to detect the presence of avian influenza genetic material is present in the clinical sample. If present, an additional rRT-PCR assay that target the presence of H5 and H7 proteins is conducted. The presence of either of either viral subtype triggers further molecular testing to confirm the presence of a HPAI or LPAI strain. Although rRT-PCR assays are scientifically robust, they are labour intensive and require expensive laboratory equipment. In addition, the process is time-consuming; samples must be transported to the laboratory followed by testing, a process which can take over 4 hours to generate results. The current approach does not give farm staff real-time accurate information about their flocks and therefore, they can not make informed decisions.

There is a growing need to develop a rapid, user-friendly and cost-effective diagnostic test kit that could be used on the field by a range of users including farm staff and veterinarians. Access to these types of test kits would help with the early and rapid detection of the disease and/or outbreaks, thus allowing farm staff to quarantine their animals quickly, ultimately helping to reduce disease spread.

References

1 Page 1 – Criteria – 066 Annual Poultry Export/Import Report – Monthly Breakdown with Prior Year Comparison – Agricultural Industry Market Information System (AIMIS)

2 FactSheet – Avian Influenza – Canadian Food Inspection Agency

▼ **Maximum grant value and travel**

Multiple grants could result from this challenge.

Phase 1:

- The maximum funding available for any Phase 1 Grant resulting from this Challenge is : $150,000.00 CAD
- The maximum duration for any Phase 1 project funded by a grant resulting from this Challenge is up to 6 months
- Estimated number of Phase 1 grants: 2

Phase 2:

- The maximum funding available for any Phase 2 Grant resulting from this Challenge is : $1,000,000.00 CAD
- The maximum duration for any Phase 2 project funded by a grant resulting from this Challenge is up to 24 months
  - Note: Only eligible businesses that have completed Phase 1 could be considered for Phase 2.
- Estimated number of Phase 2 grants: 1

Note: Selected companies are eligible to receive one grant per phase per challenge.

This disclosure is made in good faith and does not commit Canada to award any grant for the total approximate funding. Final decisions on the number of Phase 1 and Phase 2 awards will be made by Canada on the basis of factors such as evaluation results, departmental priorities and availability of funds. Canada reserves the right to make partial awards and to negotiate project scope changes.

Travel

No travel is anticipated. The kick-off meeting and final review meeting will have the flexibility of being by telephone or videoconference.

Kick-off meeting

All communication will take place by telephone or videoconference.

Progress review meeting(s)

Any progress review meetings will be conducted by telephone or videoconference.

Final review meeting

All communication will take place by telephone or videoconference.

▼ Eligibility

Solution proposals can only be submitted by a small business that meets all of the following criteria:

- for profit
- incorporated in Canada (federally or provincially)
- 499 or fewer full-time equivalent (FTE) employees *
- research and development activities that take place in Canada
- 50% or more of its annual wages, salaries and fees are currently paid to employees and contractors who spend the majority of their time working in Canada *
- 50% or more of its FTE employees have Canada as their ordinary place of work *
- 50% or more of its senior executives (Vice President and above) have Canada as their principal residence *

* Calculations must take into account and include affiliated businesses, such as parent companies and subsidiaries, that are either in or outside of Canada.

▼ Evaluation criteria

The applicant must complete the Challenge Stream Electronic Submission Form with a degree of information sufficient to enable Canada's assessment of the proposal against the criteria and the Evaluation Schema. The information must demonstrate how the proposal meets the criterion.

Part 1: Mandatory Criteria

Proposals must meet all mandatory criteria identified by achieving a "Pass" in order to proceed to Part 2. Proposals that do not meet all mandatory criteria will be deemed non-responsive and given no further consideration.

Mandatory Criteria

(Applicant's proposal must address)

Question 1 a: Scope

Describe the proposed solution and demonstrate how it responds to the challenge. Include in your description the scientific and technological basis upon which the solution is proposed and clearly demonstrate how the solution meets all of the Essential (Mandatory) Outcomes (if identified) in the Desired Outcomes section in the Challenge Notice.

Evaluation Schema (Mandatory - Pass/Fail)

Pass

The Applicant's proposed solution is clearly articulated, within the scope for the challenge and addresses all Essential (Mandatory) Outcomes (if identified) in the Challenge Notice.

Fail

The proposed solution is articulated as out of scope for the challenge.
OR
The proposal does not clearly demonstrate how the proposed solution addresses all Essential Outcomes listed in the challenge.
OR

The proposed solution is poorly described and does not permit concrete analysis.
OR
There is little to no scientific and/or technological evidence that the proposed solution is likely to meet the challenge.

Question 2: Current Technology Readiness Level (TRL)

- Indicate the current TRL of the proposed solution. (Drop Down Menu of the Challenge Stream Electronic Submission Form)
- Describe the research and development activities that have taken place to bring the proposed solution to the stated TRL.

Evaluation Schema (Mandatory - Pass/Fail)

Pass: The Applicant has demonstrated that the proposed solution is currently between TRLs 1 and 6 (inclusive), and provided justification by explaining the research and development (R&D) that has taken place to bring the solution to the stated TRL.

Fail: The Applicant has not provided sufficient evidence to demonstrate that the current TRL is between 1 to 6 (inclusive) including:

- *There is insufficient/no evidence provided for TRL judgment.*
- *The solution involves the development of basic or fundamental research.*
- *The solution is demonstrated at TRL 7 or higher.*
- *Insufficient/unclear/no justification explaining the R&D that took place to bring the solution to the stated TRL.*
- *The explanation simply paraphrases the description of a given TRL level.*

Question 3a: Innovation

Demonstrate how the proposed solution meets one or more of the ISC definitions of innovation below:

- An invention *, new technology or new process that is not currently available in the marketplace.
- Significant modifications to the application of existing technologies/components/processes that are applied in a setting or condition for which current applications are not possible or feasible.
- An improvement in functionality, cost or performance over an existing technology/process that is considered state-of-the-art or the current industry best practice.

* An "invention" is defined for the purposes of ISC as: "A manufacturing design or any other new and useful improvement that is new or novel, that is, not commonly known or not an obvious derivative of an existing way of doing things."

Evaluation Schema (Mandatory – Pass/Fail)

Pass:

The Applicant has demonstrated that the proposed solution meets one or more of the ISC definitions of innovation.

Fail:

- Applicant has not provided sufficient evidence to demonstrate that the proposed solution meets any of the ISC definitions of innovation; OR
- Applicant has demonstrated that the proposed solution is an incremental improvement, "good engineering", or a technology that would go ahead in the normal course of product development (i.e. the next version or release).

Question 3b: Advance on State of the Art

Describe in detail the competitive advantages and level of advancement over existing technologies. Where appropriate, name existing technologies as well as potential substitutes or competitors.

To demonstrate this, proposals should include the following information:

- Improvements (minor or major) over existing technologies or substitutes. Use direct comparison.
- How the proposed innovation will create competitive advantages in existing market niches or market spaces.

Evaluation Schema (Mandatory Criteria – Pass/Fail + Points)

0 points/Fail:

- The Applicant has not demonstrated that the proposed solution advances the state-of-the-art over existing technologies, including available competing solutions; OR
- The proposed solution improves minimally upon the current state of the art, though not sufficiently enough to create competitive advantages in existing market niches; OR
- The stated advancements are described in general terms but are not substantiated with specific, measurable evidence.

5 points/Pass:

- The Applicant has demonstrated that the proposed solution offers one or two minor improvements to existing technologies, including available competing solutions, that have potential to create competitive advantages in existing market niches.

12 points/Pass:

- The Applicant has demonstrated that the proposed solution offers three or more minor improvements to existing technologies, including available competing solutions, that together are likely to create competitive advantages in existing market niches; OR
- The Applicant has demonstrated that the proposed solution offers one significant improvement to existing technologies that is likely to create competitive advantages in existing market niches

20 points/Pass:

- The Applicant has demonstrated that the proposed solution offers two or more significant improvements to existing technologies, including available competing solutions that are likely to create competitive advantages in existing market niches and could define new market spaces; OR
- The Applicant has demonstrated that the proposed solution can be considered a new benchmark of state of the art that is clearly ahead of competitors and that is likely to define new market spaces

Part 2: Point-Rated Criteria

Proposals must meet the overall minimum pass mark of 50% to be deemed responsive. Proposals that do not achieve the minimum pass mark will be declared non-responsive and given no further consideration.

Point-Rated Criteria

(Applicant's proposal to address)

Question 1b: Scope

Demonstrate the scientific and technological basis of how the proposed solution addresses the *Additional Outcomes* (if identified) in the Desired Outcomes section in the Challenge Notice. If no Additional Outcomes are identified in the Challenge Notice, text entered in this section will not be considered.

If no *Additional Outcomes* are identified in the Challenge Notice, Applicants will receive 10 points.

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate that the solution will address any of the Additional Outcomes. *0 points*
ii. Information provided clearly demonstrates that the solution will address some (<50%) of the Additional Outcomes. *3 points*
iii. Information provided clearly demonstrates that the solution will address most (50% or more) of the Additional Outcomes. *6 points*
iv. Information provided clearly demonstrates that the solution will address all (100%) of the Additional Outcomes. *10 points*

Question 4: Phase 1 Science and Technology (S&T) Risks

Describe potential scientific and/or technological risks to the successful development of the proof of feasibility and how they will be mitigated in Phase 1.

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate that the Applicant has considered potential risks and mitigation strategies and/or information provided

contains significant gaps. *0 points*

ii. Information provided demonstrates that the Applicant has considered some potential risks and associated mitigation strategies but there are minor gaps in risks and/or associated mitigation strategies. *5 points*

iii. Information provided clearly demonstrates that the Applicant has sufficiently considered the risks and defined associated mitigation strategies. *10 points*

Question 5: Phase 1 Project Plan

Demonstrate a feasible Phase 1 project plan by completing the table.

- Indicate if any milestones and activities will be completed concurrently
- Indicate the estimated exit TRL at the completion of Phase 1. (Drop Down Menu of the Challenge Stream Electronic Submission Form)

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate a feasible project plan for Phase 1 and/or the project plan exceeds the maximum duration indicated in the Challenge Notice. *0 points*

ii. Project plan for Phase 1 is conceivably feasible but not clearly demonstrated and/or includes gaps. *10 points*

iii. Information provided clearly demonstrates a feasible project plan for Phase 1. *20 points*

Question 6: Phase 1 Project Risks

Describe potential project risks to the successful development of the proof of feasibility and how they will be mitigated in Phase 1.

Applicants should address the following risks, as applicable:

- Human Resources
- Financial
- Project Management
- Intellectual Property
- Other project-related risks

Note to Applicants: S&T risks should not be included in this section. Question 4 addresses S&T risks.

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate that the Applicant has considered potential risks and mitigation strategies and/or information provided contains significant gaps. *0 points*
ii. Information provided demonstrates that the Applicant has considered some potential risks and associated mitigation strategies but there are minor gaps in risks and/or associated mitigation strategies. *5 points*
iii. Information provided clearly demonstrates that the Applicant has sufficiently considered the risks and defined associated mitigation strategies. *10 points*

Question 7: Phase 1 Implementation Team

Demonstrate how the project implementation team has the required management and technological skill sets and experience to deliver the project plan for Phase 1 by completing the table. A member of the implementation team can have more than one role.

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate that the project team has the required management and technological skill sets and experience to deliver the Phase 1 project plan. *0 points*
ii. Information is provided but there are minor gaps in required management and/or technological skill sets and/or experience to deliver the Phase 1 project plan. *10 points*
iii. Information provided clearly demonstrates that the project team has the required management and technological skill sets and experience to deliver the Phase 1 project plan. *20 points*

Question 8: Inclusivity

If your business were to receive funding from Innovative Solutions Canada, describe what actions (e.g., recruitment strategy, internships, co-op placements, etc.) might be taken in Phase 1 to support the participation of under-represented groups (e.g., women, youth, persons with disabilities, Indigenous people, visible minorities) in the research and

development of the proposed solution. Each Applicant in their response to this question must focus only on describing relevant programs, policies, or initiatives that it currently has in place or would put in place to support the R&D effort in Phase 1.

Note: Do not provide any personal information of individuals employed by your company or that of your subcontractors in the response.

Evaluation Schema (Point-Rated)

i. No description and/or concrete examples of actions provided that would be taken to encourage greater participation of under-represented groups. *0 points*
ii. A description and concrete examples of actions to encourage greater participation of under-represented groups provided.*5 points*

Question 9: Phase 1 Financial Proposal

Demonstrate a realistic financial proposal for the Phase 1 project plan by completing the table.

Evaluation Schema (Point-Rated)

i. Insufficient information provided and/or information provided significantly lack credibility. Does not demonstrate a realistic financial proposal for the Phase 1 project plan. *0 points*
ii. Information is provided but some costs lack credibility and/or are unclear for the Phase 1 project plan. 7.*5 points*
iii. Information provided contains credible elements to clearly demonstrate a realistic financial proposal for the Phase 1 project plan. *15 points*

Question 10: Phase 1 Financial Controls, Tracking and Oversight

Describe the financial controls, tracking and oversight that will be used to manage the public funds throughout Phase 1. Applicants should indicate if an individual or firm will be managing the public funds and provide their credentials and/or relevant experience.

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate the Applicant's ability to manage public funds in Phase 1. *0 points*
ii. Information provided is vague and/or contains gaps. The Applicant has some controls, tracking and/or oversight in place to manage the public funds in Phase 1. *5 points*
iii. Information provided clearly demonstrates that the Applicant has strong financial controls, tracking and oversight to manage public funds in Phase 1. *10 points*

Question 11: Phase 2 Overview

Demonstrate a realistic overview for the prototype development plan if selected to participate in Phase 2.

Responses should include:

- key tasks
- estimated cost for materials
- human resources
- project risks and mitigation strategies

Note: A more detailed proposal will be requested if selected to participate in Phase 2.

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate that the Applicant has contemplated a realistic overview for the Phase 2 prototype development. *0 points*
ii. Information provided demonstrates a conceivably realistic overview for Phase 2 prototype development, however there are gaps and/or the strategy is vague. *6 points*
iii. Information provided demonstrates that the Applicant has a clear and realistic overview. *12 points*

Question 12: Commercialization Approach

Demonstrate a realistic overall commercialization approach/business model that can successfully take the technology/service to market, and how the technology/service will help you develop and sell other products.

Responses should include:

- Target markets (excluding Government of Canada)
- Non-ISC funding sources
- Transition to a commercially-ready product or service
- Any other indicators of commercial potential and commercial feasibility

Note: A more detailed proposal will be requested if selected to participate in Phase 2.

Evaluation Schema (Point-Rated)

i. Insufficient or no information provided to demonstrate that the proposed solution has commercial potential. *0 points*
ii. Some information provided to demonstrate that the proposed solution has commercial potential, however there are gaps in the commercialization approach. *6 points*
iii. A realistic commercialization approach is provided that demonstrates that the proposed solution has commercial potential. *12 points*

Question 13: Resulting Benefits to Canada

Describe the benefits that could result from the commercialization of the proposed solution. Applicants should consider the potential benefits using the following three categories and provide justification for each claim:

- Innovation Benefits: Expected contribution towards the enhancement or development of new industrial or technological innovations within your firm. Responses could include: potential spillover benefits, creation of intellectual property, impact on productivity of the new technology, etc.
- Economic Benefits: Forecasted impact on the growth of Canadian firms, clusters and supply chains, as well as its expected benefits for Canada's workforce. Responses could include: number of jobs created, number of high-paying jobs, investment in Canada's economy, etc.
- Public Benefits: Expected contribution to the broader public to the degree that the solution is expected to generate social, environmental, health, security or other benefits to Canada. Responses could include: solution-related environmental benefits, solution-related accessibility benefits, and solution-related impact on Indigenous communities.

Evaluation Schema (Point-Rated)

i. Innovation Benefits

Benefit not identified or insufficient claim of benefit. *0 points*

Benefit has marginal increment or limited justification. *1 point*

Benefit is significant and well justified. 2 *points*

ii. Economic Benefits

Benefit not identified or insufficient claim of benefit. *0 points*

Benefit has marginal increment or limited justification. *1 point*

Benefit is significant and well justified. *2 points*

iii. Public Benefits.

Benefit not identified or insufficient claim of benefit. *0 points*

Benefit has marginal increment or limited justification. *1 point*

Benefit is significant and well justified. *2 points*

▼ Questions and answers

▶ Question : The Essential Outcomes state that the solution should be of a size and weight that makes the unit portable by a single individual for field use. Should the unit include additional equipment?

All incoming questions regarding this specific challenge should be addressed to solutions@ised-isde.gc.ca.

All enquiries must be submitted in writing no later than ten calendar days before the Challenge Notice closing date. Enquiries received after that time may not be answered.

You can also consult the Frequently asked questions about the Innovative Solutions Canada Program.

A glossary is also available.

Date modified:
2025-01-08

Latest bird flu situation

We are currently responding to cases avian influenza in domestic birds across Canada. Anyone with birds must practice good biosecurity habits to protect poultry and prevent disease.

To date, the most common avian influenza virus in domestic birds has been highly pathogenic avian influenza (HPAI), subtype H5N1.

- On November 8, 2024, the CFIA confirmed the presence of the HPAI H5N2 subtype in poultry in British Columbia.
- On November 25, 2024, the CFIA detected low pathogenic avian influenza (LPAI), subtype H5, in Quebec.
- The HPAI virus found in U.S. dairy cattle has not been detected in birds or any other animals in Canada.

Avian influenza is not a food safety concern. There is no evidence to suggest that eating cooked poultry or eggs could transmit the virus to humans.

Most requested

- CFIA's Response to Highly Pathogenic Avian Influenza (HPAI) on British Columbia Ostrich Farm
- Biosafety advisory: Avian influenza A(H5N1)
- Animals susceptible to H5N1 HPAI
- Information for travellers: Restrictions from the United States
- Restrictions on poultry exports
- Avian influenza in wild birds
- Countries recognized as being free from HPAI
- Statement: Government of Canada provides an update on HPAI

This is Exhibit "J" referred to in the affidavit of Katrina Jonas sworn before me at White Rock this 30 day of January 2025

Services and information

HPAI in livestock
Information about HPAI in dairy cows in the U.S. and guidance for producers and veterinarians.

Status by province

Avian influenza detections by province and estimated number of infected birds.

Moving flocks and poultry products through control zones

Map of affected areas, permits and conditions.

Permits and conditions

What is required for the movement of birds and by-products through a control zone.

Investigations and orders

Current and recent investigations in each province.

Infected and high risk premises

What to expect, compensation, cleaning and disinfection.

HPAI science and research

Science and research related to the prevention, detection, response and management of HPAI in animals.

Facts about avian influenza (bird flu)

General information about bird flu and reducing the spread of the disease.

HPAI dashboards

Data on HPAI in Canadian domestic birds and wildlife.

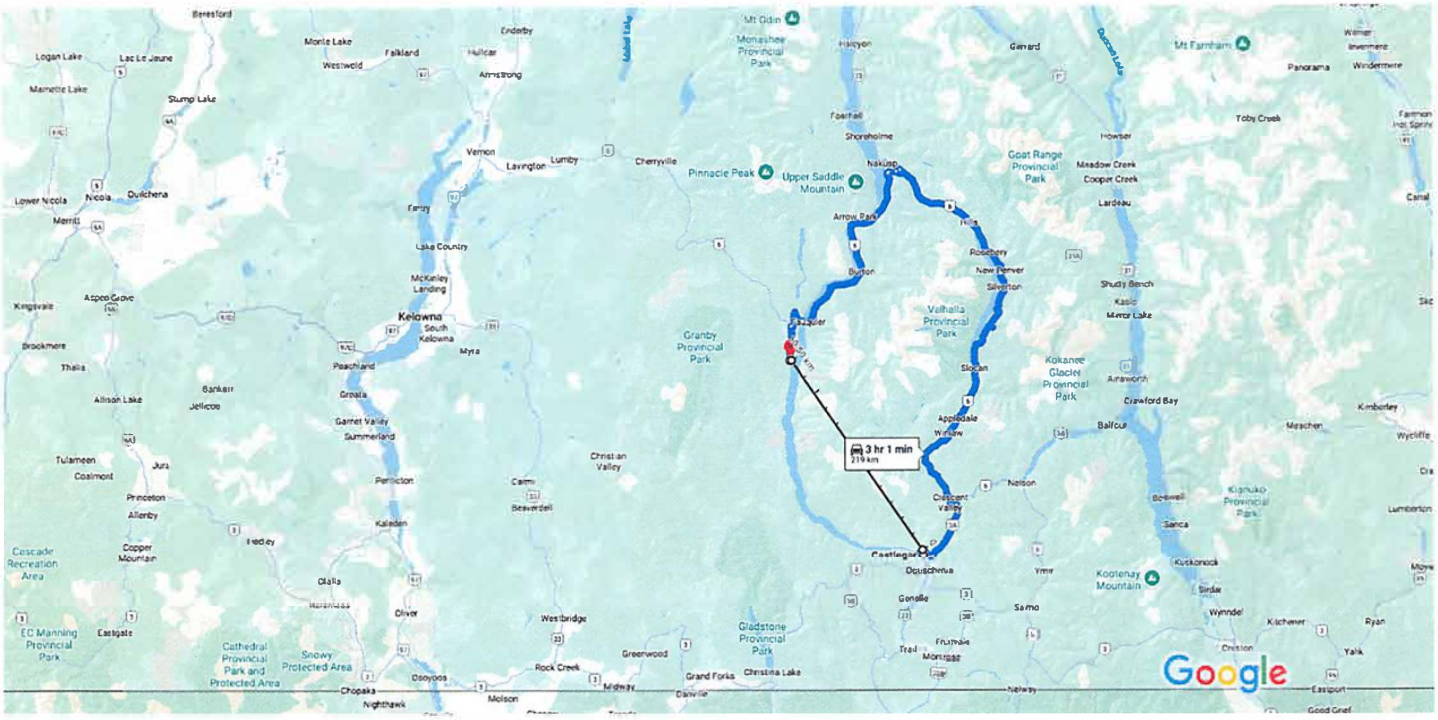
Date modified:

2025-01-27

Google Maps

Castlegar, British Columbia to Edgewood, British Columbia V0G 1J0

Drive 219 km, 3 hr 1 min



Map data ©2025 Google 20 km

via BC-6 N and BC-6 — 3 hr 1 min
Fastest route, the usual traffic — 219 km
⚠ This route includes a ferry.

via BC-6 N — 3 hr 2 min
221 km

This is Exhibit "K" referred to in the affidavit of Kaetrina Jones sworn before me at White Rock this 30 day of January 20 25

Explore Edgewood

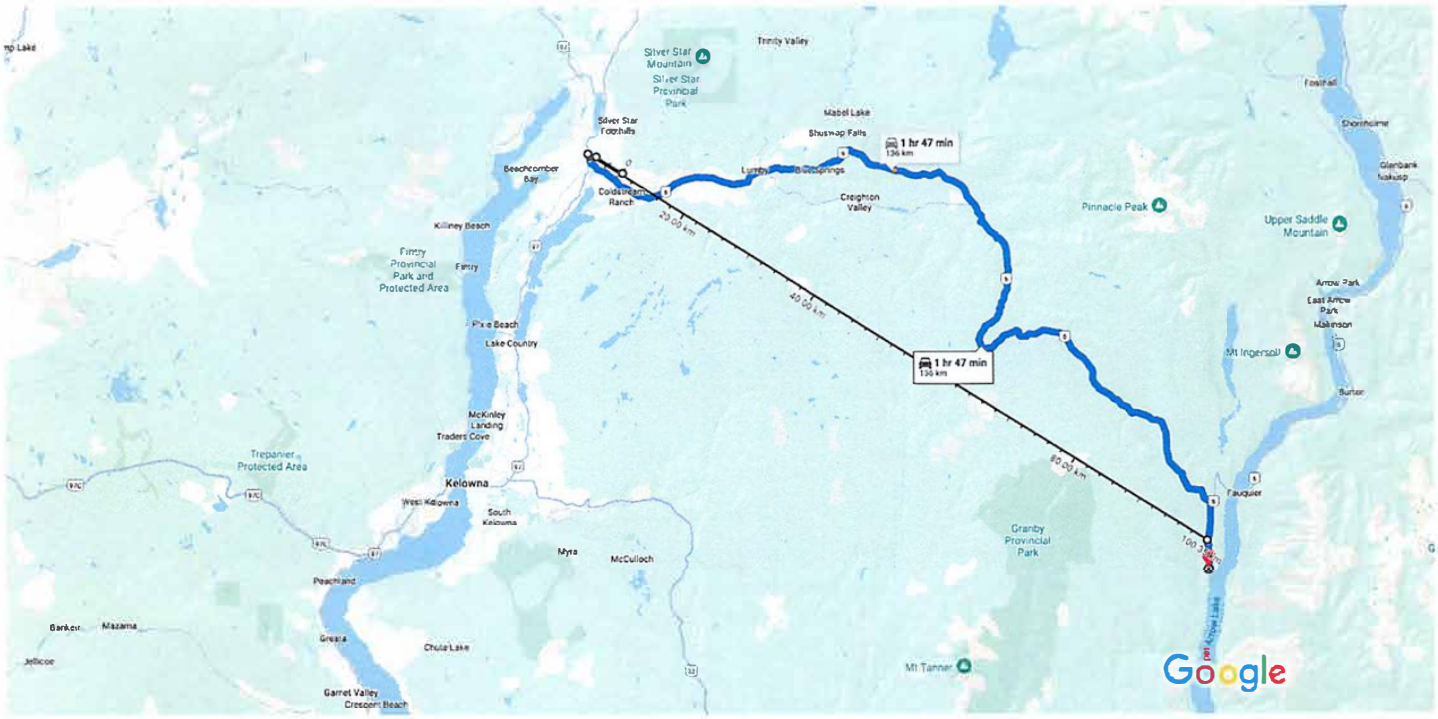
Restaurants Hotels Gas stations Parking Lots More

Measure distance
Total distance: 60.50 km (37.60 mi)

Google Maps

Vernon, British Columbia to Edgewood, British Columbia V0G 1J0

Drive 136 km, 1 hr 47 min



Map data ©2025 Google 10 km

via BC-6 Best route	1 hr 47 min 136 km
via BC-6 and Edgewood Rd/Inonoaklin Valley Rd	1 hr 47 min 136 km

This is Exhibit "L" referred to in the affidavit of Katrina Jones sworn before me at White Rock this 30 day of January 2025

Explore Edgewood

Restaurants Hotels Gas stations Parking Lots More

Measure distance
Total distance: 100.35 km (62.35 mi)

This is Exhibit "M" referred to in the
affidavit of Katrina Jones
sworn before me at White Rock
this 30 day of January 2025

ORDER NO. / N° DE COMMANDE

DEPT. / SERV.

DATE May 23/2021

SOLD TO / VENDU À: Mountain Man Dog Sled Adventures

ADDRESS / ADRESSE: Chris & Taryn Schwanke
3280 McGillivray Creek Rd.
Heffley Creek, BC. V0E 1Z1

SHIP TO / EXPÉDIER À: SELLER

ADDRESS / ADRESSE: Universal Ostrich Farm Inc
301 Langille Rd.
Edgewood, BC V0G 1J0

SHIPPING DATE / DATE D'EXPÉDITION	VIA	TERMS / CONDITIONS	TAX EXEMPTION NO. / N° DE TAXE	SOLD BY / VENDU PAR

QUANTITY ORDERED / QUANTITÉ	DESCRIPTION	PRICE / PRIX	AMOUNT / MONTANT
1	Rooster 444 WT - 4 years old. Blue/Black	7500	
1	Rooster - BT- 5 years old. Blue	7500	
1	Hen - ~~234~~ 232 BT - 5 years old Blue/Black	7500	
1	Hen 233. BT- 4 years old Black	7500	
1	Hen 231 BT- 4 years old Black.	7500	
1	Hen ~~230~~ 234 BT- 4 year old. - Blue/Black	7500	
	Deposit Paid - $22,500 April/202		
	Final Payment Due immediately. $22.500 May 23/2021		
	Paid. NKob Incubator $4500 + Hatcher.	$4500	
		TVH - HST TPS - GST	
		PST-TVP	49 500
SALESPERSON / VENDEUR	DATE	TOTAL	49.50

SALES ORDER
FORMULAIRE DE VENTE