29 MAY 2024

TO		**FROM**	
Client:	[redacted]	**Name:**	[redacted]
Attention:	[redacted]	**Section:**	Science, Technology, Environment and Resource
Tel:		**Tel:**	[redacted]
Email:	[redacted]	**Email:**	[redacted]
		TRIM Reference:	

Avian influenza outbreak

Thank you for your question relating to the avian influenza outbreak detected in Victoria, received on 28 May 2024 by email.

You have requested assistance responding to the following constituent questions, regarding the outbreak:

1. What specific tests were performed to establish that this outbreak was due to H7N3 avian flu
2. Where have those test results been documented and/or uploaded
3. Have the biosample sequences been uploaded to GenBank
4. How many tests were performed and how many chickens were found to have tested positive
5. If PCR was the primary testing modality, what Ct value were the tests performed (or claimed positive) at
6. Who made the decision to cull 500,000 birds.

You requested this information ASAP.

Caveat

This topic of national animal diseases management complex and, in the time available, this brief cannot be considered fully comprehensive. With more time, we could provide further information.

Avian influenza background

This section is reproduced from previous work undertaken by my colleague Dr Emily Hanna. Note, in the time available the links and references used in this section have not been updated.

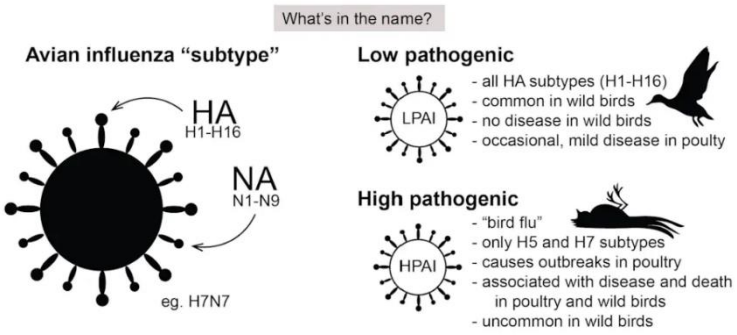
Avian influenza (AI; also known as bird flu) is a worldwide infectious bird disease of varying severity. It has been recorded in over 140 species of domestic and wild birds, including numerous poultry species, such as chicken, turkeys, ducks and ostriches. AI has also been identified in mammal species including pigs and domestic cats. Humans are susceptible, although 'there is minimal risk of people being affected by AI viruses through normal contact with birds' in Australia.[1]

Avian influenza, like human influenza, is caused by numerous different viruses. Bird flu viruses are all Type A viruses (flu viruses come in types A, B, C and D: A and B types cause the seasonal flu epidemics in humans, C types are milder and less common in humans, and D types are not known to affect humans, mainly infecting cattle).[2] AI viruses are classified into subtype by identification of 2 surface proteins, Hemagglutinin (HA), which currently has 16 identified subtypes H1–H16, and Neuraminidase (NA), which currently has 9 subtypes N1–N9. For example, H5N1 bird flu has the HA subtype H5 and the NA subtype N1. Numerous HA and NA subtype combinations occur.

Bird flu strains are also designated as either highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI), depending on severity of the disease and its potential to cause death in poultry.[3] Figure 1 shows how AI viruses are classified. AI viruses evolve constantly, creating new strains.[4] It is common for multiple strains to co-exist globally.[5]

1. 'Avian influenza (bird flu)', DAFF.
2. 'Avian influenza (bird flu)', DAFF; 'Influenza (Seasonal)', World Health Organization, 3 October 2023.
3. 'Avian influenza (bird flu)', DAFF.
4. Wildlife Health Australia National Avian Influenza Wild Bird Steering Group, *Technical Issue Update - Global High Pathogenicity Avian Influenza Events version 3*, (Wildlife Health Australia, 2023).
5. Michelle Wille, Victoria Grillo, Silvia Ban de Gouvea Pedroso, Graham W. Burgess, Allison Crawley, Celia Dickason, Philip M. Hansbro et al., 'Australia as a global sink for the genetic diversity of avian influenza A virus', *PLoS Pathogens* 18, no. 5 (2022): e1010150; Wildlife Health Australia National Avian Influenza Wild Bird Steering Group, *Technical Issue Update - Global High Pathogenicity Avian Influenza Events version 3*.

Figure 1 Classification of avian influenza viruses



Avian influenza viruses are classified in two ways - the first is based on the HA and NA subtypes, and the second is based pathogenicity. Michelle Wille

Source: Michelle Wille and Stacey Lynch, '[Nearly half a million poultry deaths: there are 3 avian influenza outbreaks in Victoria. Should we be worried?]', *The Conversation*, 7 October 2020.

Although most AI strains have little effect on wild birds and poultry, some strains of LPAI can evolve into an HPAI in poultry. The H5 and H7 subtypes have the highest potential to develop into an HPAI. HPAI can cause severe disease and high rates of mortality in the poultry, as well as potentially spreading back to wild bird populations. This can sometimes result in death and severe disease in the wild birds in addition to increasing the chances of spread of the HPAI to other domestic poultry populations via the infected wild birds.[6]

1. What specific tests were performed to establish that this outbreak was due to H7N3 avian flu

I note that there have been 2 different strains of high pathogenicity avian influenza detected in Victoria. The Australian Government's 'Avian influenza' webpage provides the following information concerning the 2 recent outbreaks of avian influenza in Victoria:

> On 22 May 2024, high pathogenicity avian influenza (HPAI) H7N3 was detected on a poultry farm near Meredith, Victoria.
>
> On 24 May 2024, a second Victorian poultry farm near Terang was confirmed to have HPAI H7N9, due to Agriculture Victoria's tracing activities. The properties are commercially linked and approximately 110km apart.
>
> Testing at the CSIRO's Australian Centre for Disease Preparedness laboratory confirmed that these strains are genetically related to viruses previously detected in Australian wild birds. The viruses are **not the H5 strain currently causing concern globally**.
>
> There is **no connection** between these detections of H7N3 and H7N9 in Victorian poultry and the recent detection of H5N1 avian influenza in a person, who recently returned from travel overseas. [emphasis in original]

The following information on testing has been taken from the latest version (version 5.2) of the national *AUSVETPLAN response strategy for avian influenza*, which is currently still a working draft.

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6. 'Avian influenza (bird flu)', DAFF.

The strategy 'sets out the nationally agreed approach to avian influenza outbreaks in Australia', and is part of the Australia's nationally agreed arrangements in place to respond to animal disease incidents and outbreaks. Sections 2.5.4 and 2.5.5 of the strategy outlines the laboratory tests and diagnosis process in Australia (pp. 32–36).

Regarding tests, the strategy states (pp. 32–33):

> Because clinical and pathological changes are not definitive for AI, diagnosis must be confirmed by isolation of the virus or by characterisation of fragments of its genome (WOAH 2022). Relevant laboratory tests should be performed to exclude Newcastle disease and bacterial septicaemias from the differential diagnosis, particularly to identify mixed infections with less pathogenic forms of AI.
>
> If an outbreak is confirmed to be caused by an HPAI virus, this agent may also be classified as a security sensitive biological agent (SSBA), to which regulatory requirements (eg for handling and reporting) may apply. This may be the case if there is proven infection of humans and there is an outbreak of human disease. However, emergency situations, including emergency animal disease (EAD) outbreaks, may be exempted from some SSBA regulatory requirements, although this is not automatic. Clarification should be sought from the SSBA-responsible officer at the facility concerned.
>
> …
>
> **Transport of specimens**
>
> Specimens should be submitted in accordance with agreed state or territory protocols. Specimens should initially be forwarded to the state or territory laboratory for appropriate analysis, and assessment of whether further analysis will be required by the CSIRO Australian Centre for Disease Preparedness (CSIRO-ACDP), Geelong.
>
> If the state or territory laboratory deems it necessary, duplicate samples of the specimens should be forwarded to CSIRO-ACDP for emergency disease testing, after the necessary clearance has been obtained from the chief veterinary officer (CVO) of the state or territory of the suspect case, and after the CVOs of Victoria and Australia have been informed about the case and the transport of the specimens to Geelong (for the first case). Sample packaging and consignment for delivery to CSIRO-ACDP should be coordinated by the relevant state or territory laboratory.

Regarding diagnosis, the strategy states (pp. 33–34):

> The initial approach to AI diagnosis is screening by real-time PCR. Primary screening uses a pan-influenza A assay, as well as specific H5 and H7 assays. Further subtype-specific assays may also be run, if required. Any positive isolates are further characterised by culture in eggs and further molecular analysis. Analysis of viral genetic sequence data allows assessment of pathogenicity (see 'Agent characterisation', below), as well as more-detailed phylogenetic analysis.
>
> Isolates obtained from egg culture are identified antigenically by hemagglutination inhibition (HI), as well as with molecular tools.

Table 1 has been reproduced from the strategy and details the laboratory tests that are currently available at CSIRO-ACDP for the diagnosis of avian influenza. Figure 1 shows the CSIRO-ACDP diagnostic testing approach.

Table 1 Laboratory tests available at CSIRO-ACDP for the diagnosis of avian influenza

Test	Specimen required	Test detects	Time taken to obtain result
Agent detection			
Real-time PCR[a]	Swabs, tissues	Type A influenza, H5 and H7 subtypes, some LPAI subtypes	<1 day
Immunohistochemistry	Formalin-fixed tissues	Viral antigen	2 days
Virus isolation in embryonated eggs	Swabs, tissues	Virus	2–10 days
Agent characterisation			
PCR and sequencing	Swabs, tissues or virus isolate	Viral RNA	2–3 days
Antigenic subtyping (HI)	Virus isolate	Specific HA and NA antigens	1–4 days
Intravenous pathogenicity index	Virus isolate	Virulence of virus	2–10 days
Serology			
ELISA[a]	Serum	Antibody (influenza A)	1 day
HI	Serum	Antibody (specific HA types)	1 day

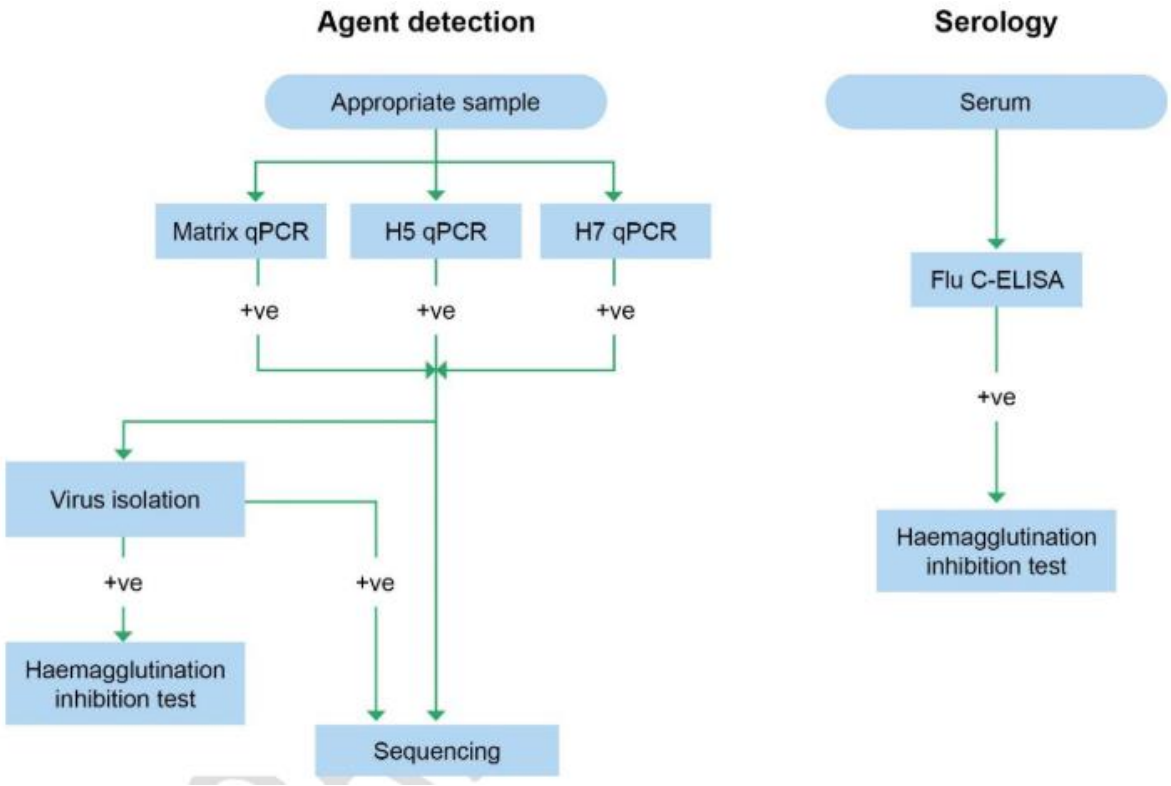
ELISA = enzyme-linked immunosorbent assay; HA = haemagglutinin; HI = haemagglutination inhibition; LPAI = low pathogenicity avian influenza; NA = neuraminidase; PCR = polymerase chain reaction

a Test also supported as part of the LEADDR network.

Source: Information provided by the then CSIRO-AAHL, 2023 (refer to CSIRO-ACDP for most up-to-date information).

Source: Animal Health Australia, *AUSVETPLAN response strategy for avian influenza*, (version 5.2, working draft), (Canberra: Animal Health Australia, 2023), 35.

Figure 1 Current approach to aviation influenza diagnostic testing at CSIRO-ACDP



ELISA = enzyme-linked immunosorbent assay; HA = haemagglutinin; HI = haemagglutination inhibition; LPAI = low pathogenicity avian influenza; NA = neuraminidase; PCR = polymerase chain reaction
Source: Animal Health Australia, *AUSVETPLAN response strategy for avian influenza*, (version 5.2, working draft), (Canberra: 2023), 34.

Pages 35–36 of the strategy provide the following further information concerning testing:

> Agent characterisation: tests for virus subtype AI viruses are subtyped on the basis of the sequence of the HA and NA genes, as well as their HA and NA antigens. Details of tests for subtyping (PCR and sequencing, and antigenic subtyping) are in Table 2.1.
>
> **Agent characterisation**
>
> *Tests for pathogenicity*
>
> The pathogenicity of an influenza virus isolated from a bird is generally assessed by sequencing the part of the gene encoding the cleavage site of the HA protein of the virus (molecular pathotyping). In some circumstances it is necessary to verify high or low pathogenicity of a virus isolate using a chicken pathogenicity test (intravenous pathogenicity index) (WOAH 2022).
>
> For a timely diagnosis, molecular pathotyping is the preferred method of determining the pathogenicity of an AI virus in Australia. Once an outbreak virus has been characterised, virus detection and virus isolation are generally sufficient to confirm further virulent infections.
>
> *Tests for previous infection*

Evidence of previous AI virus infection can be obtained by testing for influenza A group-specific antibody using an ELISA, or by testing for subtype-specific antibody to the HA or NA antigens using an HI test or ELISA, respectively.

Use of serology for AI testing has several limitations:

- HI testing at a single point in time does not provide an indication of recent infection. Repeat blood samples collected 2–3 weeks apart may allow further interpretation of the HI results if a change in titre can be demonstrated.
- Positive serology results could indicate exposure to infection before the current outbreak situation.
- Positive serology results can indicate that the bird had exposure to an antigenically similar virus, and not the specific virus present in the outbreak situation.

Field tests

Currently, no field tests are approved for use during an EAD response involving AI in Australia.

2. Where have those test results been documented and/or uploaded

Australia is required to notify the World Organisation for Animal Health (WOAH) – the intergovernmental organisation that supports and promotes animal disease control – when an influenza A virus infection is determined to be HPAI (p. 42). There are other criteria for avian influenza infections that, if met, also require WOAH to be notified (see p. 42 of the *AUSVETPLAN response strategy for avian influenza*).

The WOAH maintains a 'global reference platform for the publication of official data on epidemiologically important diseases in domestic and wild animals', called the World Animal Health Information System (WAHIS). The WAHIS webpage states:

> When an important epidemiological event pertaining to terrestrial or aquatic animals occurs in a WOAH Member, the Member must inform WOAH by sending an immediate notification comprising the reason for notification, the disease name, the affected species, the geographical area affected, the control measures applied and any laboratory tests that have been carried out or are in progress.
>
> …
>
> After having informed WOAH of a significant epidemiological event by means of an immediate notification, the Member must send weekly follow-up reports so that the event can be monitored as it evolves. In all cases, the Member must submit a final report to notify either that the event has been resolved or that the disease has become endemic. In both cases, the Member will continue to submit information in its six-monthly reports if the disease is on the WOAH List.

The 2 outbreaks events in Victoria have been lodged on the WAHIS dashboard. See the event (no. 5683) profile for the H7N3 outbreak event and the event (no. 5687) profile for the H7N9 outbreak.

The *AUSVETPLAN response strategy for avian influenza* also states (p. 42):

> For domestic purposes the finding of any strain of HPAI or LPAI virus is notifiable to the chief veterinary officer (CVO) of the state or territory in which the finding is made.

The *AUSVETPLAN management manual laboratory preparedness* outlines the roles and responsibilities of diagnostic testing laboratories during a national emergency animal disease (EAD) response. Under 'Chapter 4: Responsibilities of laboratories', the manual states: 'All states and territories have policies and regulations that restrict testing for EADs to certain laboratories, place conditions on testing at these laboratories and/or prescribe procedures for releasing results' (p. 18). Section 4.5 outlines the laboratory result reporting responsibilities (p. 19) and 'Chapter 6: Communications' outlines the communication guidelines and procedures during an EAD response (pp. 24–27).

There may also be further information in the 2 Control Centres Management Manuals – which can be viewed by selecting 'Management manuals' option, under the 'Browse AUSVETPLAN publications' heading – which outlines the 'management structure and an information flow system for handling an EAD outbreak at national, state/territory and local levels'.

3. Have the biosample sequences been uploaded to GenBank

In the time available, we have been unable to determine if the virus sequences have been added to the GenBank database.

4. How many tests were performed and how many chickens were found to have tested positive

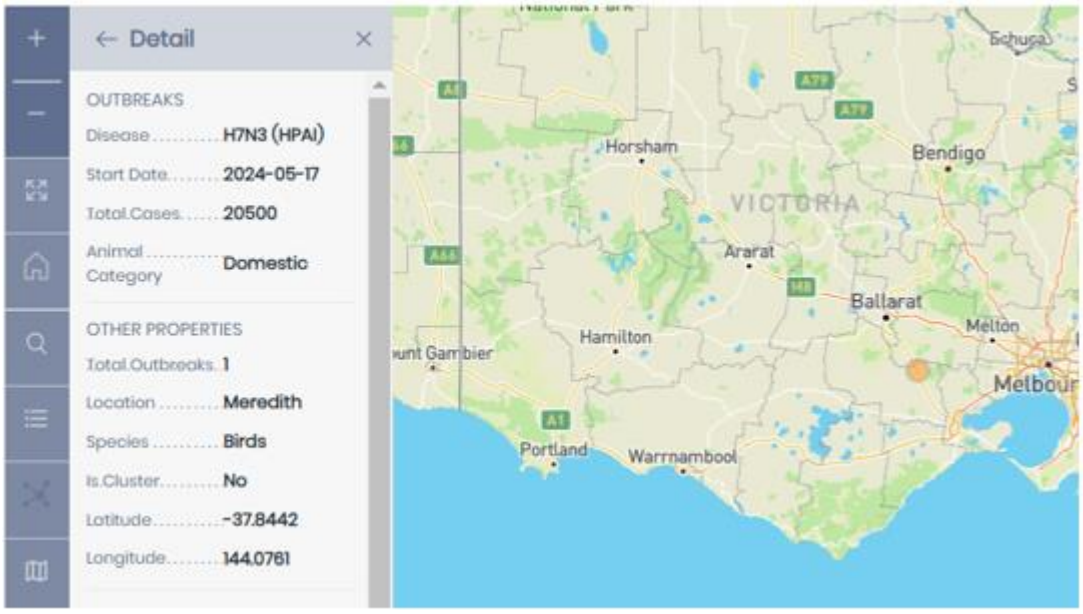
We have been unable to locate specific test result data concerning the outbreak event, however, the event profiles on the WAHIS do provide some information on the number of cases recorded for each event. It is also not immediately clear whether the case numbers are equivalent to the number of positive test result that have been recorded.

HPAI H7N3 outbreak event

The 'Quantitative Data' tab of the WAHIS event (no. 5683) profile for the HPAI H7N3 outbreak event in Victoria provides the following figures: Of the 412,000 susceptible birds, there have been 7,500 cases recorded and 3,000 deaths. Note the 'death' figure does not appear to be related to the number of birds that were intentionally killed or disposed of to contain the disease.

I note that if you view this event on the WAHIS interactive map, the number of total cases for this outbreak is listed as 20,500 (Figure 2). In the time available, I have been unable to investigate why the total case figure for the outbreak is higher when viewed via the interactive map.

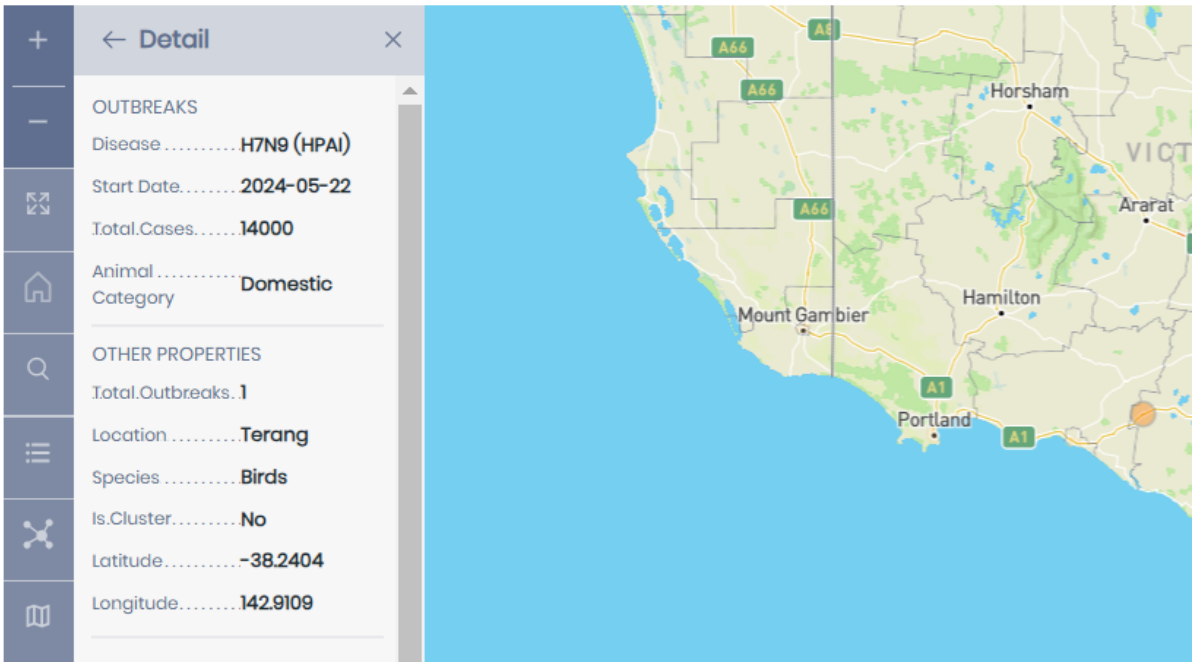
Figure 2 WAHIS interactive map details for H7N3 outbreak event (no. 5683)



HPAI H7N9 outbreak event

The 'Quantitative Data' tab of the WAHIS immediate notification event (no. 5687) report for the H7N9 outbreak records 160,000 susceptible birds, 14,000 cases and 300 deaths. The number of cases on the interactive map for this event is the same as the profile (Figure 3).

Figure 3 WAHIS interactive map details for H7N9 outbreak event (no. 5687)



5. If PCR was the primary testing modality, what Ct value were the tests performed (or claimed positive) at

In the time available, we have been unable to locate this information.

6. Who made the decision to cull 500,000 birds

The state and territory government are responsible are responsible 'for animal health matters within their boundaries,' including matters such as 'disease surveillance and control'. As noted In the Animal Health Australia *Producer factsheet* (updated 24 May 2024):

> The state/territory government will establish declared areas as part of their response to prevent the spread of AI. Depending on their proximity to the disease, premises will be located in a restricted area, control area or outside area. There may be multiple restricted and control areas within the state/territory. The category your property/premises falls into will determine which disease control measures may apply.

Control measure include 'destruction of poultry that are infected or are a high disease risk; high risk things that can't be decontaminated might also be destroyed'. See also section 4.3 'Control and eradication policy' of the *AUSVETPLAN response strategy for avian influenza* for further information on how this control measure is implemented (pp. 47–50).

See also:

- the Victoria Government Gazette *Order Declaring a Restricted Area in Relation to Avian Influenza*, which establishes a restricted area around the properties affected
- 'High pathogenicity avian influenza (HPAI)' Agriculture Victoria, which provides an update on the cases in Victoria (to 23 May 2024).

Feedback

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