

## Prevention and Control of Avian Influenza in Singapore

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### Abstract

The highly pathogenic avian influenza (HPAI) H5N1 virus was first detected in 1996 in Guangdong, China. Since 2003, H5N1 outbreaks have been reported in parts of Asia, Europe, the Middle East, and Africa. It is currently entrenched among poultry in parts of Asia and poses a major challenge to animal and human health. Singapore is free from HPAI. Given Singapore's need to import food, the Agri-Food and Veterinary Authority (AVA) has adopted a pro-active risk management system to prevent the introduction of HPAI. AVA's approach may be described as a multi-layered control strategy for the prevention and control of HPAI. The strategy includes control measures at source, border control measures, local control measures and emergency preparedness.

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### Introduction

Avian influenza (AI) or "bird flu" is a highly infectious disease of birds. AI viruses are negative single-stranded enveloped RNA viruses that belong to the influenza A genus of the *Orthomyxoviridae* family. AI viruses may be further divided into subtypes on the basis of the antigenic properties of their haemagglutinin (H1-H16) and neuraminidase (N1-N9) surface glycoproteins. All highly pathogenic AI viruses that cause generalised rather than respiratory disease belong to either the H5 or H7 subtypes.<sup>1</sup> The most virulent form of AI is known as highly pathogenic avian influenza (HPAI). This is a highly contagious disease of domestic fowl that was first identified as a serious disease of poultry by an Italian scientist, Edoardo Perroncito, in 1878.<sup>2</sup>

The greatest variety of AI viruses has been isolated from wild birds particularly from waterfowl belonging to the orders *Anseriformes* (ducks, geese and swans) and *Charadriiformes* (gulls and shorebirds).<sup>2</sup> Migratory waterfowl of the world are the natural reservoirs of AI viruses of all known subtypes.<sup>3</sup> Available evidence suggests that each of the 16 H and 9 N subtypes combinations exist in harmony with their natural hosts, cause no overt disease and are shed predominantly in the faeces.<sup>3</sup>

AI viruses of all 16 H subtypes can cause low pathogenicity avian influenza (LPAI) in susceptible birds.<sup>1</sup> This is usually a mild respiratory disease with low mortality rates in poultry. In contrast, HPAI is a systemic disease with high mortality rates approaching 100% in many gallinaceous birds.<sup>1</sup> Current theories suggest that HPAI viruses emerge from H5 and H7 subtypes of low pathogenicity by mutation<sup>4,5</sup> although there must be more than one mechanism by which this occurs.<sup>6</sup> It appears that such mutations occur only after the viruses have moved from their natural wild bird hosts to poultry. However, the mutation to virulence is unpredictable and may occur soon after introduction to poultry or after the LPAI virus has circulated for several months.<sup>1,5</sup> For this reason, the World Organization for Animal Health (OIE) has defined LPAI viruses of the H5 and H7 subtypes as low pathogenicity notifiable avian influenza (LPNAI) viruses.<sup>7</sup> Highly pathogenic AI viruses are defined as highly pathogenic notifiable avian influenza (HPNAI) viruses. These influenza viruses have important trade implications and are made notifiable to OIE.

Before 1997, there was no evidence to indicate that H5 AI viruses could infect humans and cause fatal disease.<sup>8</sup> The H7 influenza viruses were known to cause conjunctivitis in humans,<sup>9,10</sup> and serologic studies provided evidence of subclinical human infection with the subtypes prevalent in

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avian live poultry markets.<sup>11</sup> The precursor of the H5N1 AI virus that spread to humans in 1997 was first detected in 1996 in Guangdong, China.<sup>12</sup> In the H5N1 outbreak in Hong Kong in 1997, the AI virus infected 18 people resulting in 6 deaths and the total depopulation of the poultry population of 1.3 million birds.<sup>13</sup>

The current H5N1 outbreak that started in Asia in 2003 is unprecedented in scale and geographic distribution. The H5N1 AI viruses are now panzootic across 3 continents leading to huge economic losses and have also been transmitted to humans.<sup>13</sup> As at 28 May 2008, the World Health Organization (WHO) has recorded a total of 383 human cases of H5N1 infection with 241 deaths.<sup>14</sup> The expansion of intensive poultry husbandry is likely to be facilitating the increased frequency and scale of HPAI outbreaks. Furthermore, the present commercialised large-scale poultry industry is now shipping poultry and poultry products over long distances, facilitating the transmission of infection.<sup>13</sup>

### Keeping HPAI Out of Singapore

Singapore is free from HPAI. Given the devastating consequences of an HPAI H5N1 outbreak, the Agri-Food and Veterinary Authority of Singapore (AVA) has adopted a series of measures to keep the AI virus out of Singapore. However, Singapore is a small city-state with little agricultural land that needs to import the bulk of its food. Therefore, the AVA has adopted a pro-active risk management system to balance its control measures with the need to import food. AVA's approach may be described as a multi-layered control strategy for the prevention and control of HPAI. The strategy consists of several layers of control measures as follows:

- a) Control Measures at Source;
- b) Border Control Measures;
- c) Local Control Measures; and
- d) Emergency Preparedness

#### Control Measures at Source

##### *Prohibiting Import from HPAI Infected Countries*

Singapore imports a variety of food from overseas sources. These are either shipped in by air or sea or trucked through land checkpoints. Under the Animals and Birds Act, the import of any poultry, bird, egg or avian product requires a permit from the AVA. Singapore uses this import requirement as its first line of control against HPAI. AVA officers frequently scan media reports, Food and Agricultural Organization (FAO) and OIE websites and reports for any occurrence of HPAI outbreaks. Where outbreaks are reported, AVA takes immediate steps to suspend import of poultry, birds and avian products from the infected country.

##### *Establishing Disease-Free Zones*

Malaysia is an important source of live poultry and table eggs. Accredited poultry farms in Malaysia are allowed to export poultry and eggs to Singapore. In September 2004 when an outbreak of HPAI was reported in the Malaysian state of Kelantan, AVA suspended all imports of poultry, eggs and ornamental birds from Malaysia. This disrupted the supply and caused a shortfall of poultry and eggs in Singapore. To ensure safety of poultry and egg supplies at source and prevent frequent disruptions to our supply, AVA worked closely with its Malaysian counterpart, the Department of Veterinary Services (DVS), to create Disease-Free Zones (DFZs) in the states of Johor, Perak, Selangor, Malacca, and Negri Sembilan. As an added level of safety all accredited poultry farms are located in DFZs. The DFZs, together with the introduction of enhanced control and surveillance programmes, enabled exports to resume in January 2005 without compromising animal or public safety.

The rationale for establishing and maintaining the DFZs is to allow the export of poultry and eggs from the DFZs to continue should there be another HPAI outbreak in Malaysia outside the DFZs. Subsequently, when Malaysia reported an outbreak of HPAI in chickens in Sungei Buloh, Selangor on 2 June 2007, Singapore was able to continue import of poultry products from the DFZs of Johor, Malacca, Negri Sembilan and Perak.

The DFZs are consistent with the OIE guidelines to zoning and compartmentalisation.<sup>7,15</sup> Zoning and compartmentalisation are procedures implemented by a country with a view to defining subpopulations of different animal health status within its territory for the purpose of disease control and/or international trade.<sup>15</sup> Whilst zoning applies to an animal subpopulation defined on a geographical basis (using natural, artificial or legal boundaries), compartmentalisation applies to a subpopulation with distinct health status under a common biosecurity management system.<sup>15</sup>

Both AVA and DVS agreed to a set of conditions for establishing and maintaining a DFZ. These include:

- Clearly defined boundaries for the DFZs (these follow existing state boundaries);
- States to legislate for control of movement of poultry and poultry products in and out of the DFZ (using a system of permits and health certificates, border patrols and inspections);
- States to maintain strict biosecurity measures at all accredited poultry farms located within the DFZs;
- States to prohibit AI vaccination of poultry in the DFZs to prevent the masking of an outbreak should one occur;
- States to draw up emergency response plans (to prepare

for an HPAI outbreak in the DFZ) and to undertake to inform AVA immediately of any outbreak.

### Border Control Measures

AVA officers inspect all imports of poultry, birds, eggs and avian products at the port of entry. Poultry and birds are inspected for any overt signs of HPAI. In particular, AVA inspects all consignments of poultry from Malaysia. Each consignment is accompanied by a health certificate issued by DVS. In addition, all consignments of ducks must be tested negative for AI by polymerase chain reaction (PCR) before they can be exported to Singapore. Randomly selected consignments of poultry and eggs are also sampled and tested for HPAI. During any HPAI alerts, AVA carries out enhanced inspection and surveillance at the checkpoints.

AVA also works closely with the Immigration and Checkpoints Authority (ICA) to curb smuggling especially of birds and avian products, at borders and checkpoints.

### Local Control Measures

AVA employs a variety of local control measures against HPAI. These include biosecurity, biosegregation, surveillance, vaccination, removal of backyard poultry, improvement of diagnostic laboratory capability and public education. However, the corner stones for AVA's control measures are biosecurity and enhanced surveillance.

#### Biosecurity

Biosecurity is considered the most important tool to prevent and control AI.<sup>1,16</sup> The key is to keep migratory wild birds (especially water fowl) away from poultry and commercial bird breeding operations. AVA emphasises biosecurity at all local poultry farms, poultry slaughterhouses, bird holding and breeding premises, zoological gardens and bird parks.

AVA defines biosecurity measures as measures to keep disease (specifically HPAI) out of local poultry farms, slaughterhouses and bird breeding premises. AVA has imposed strict biosecurity measures for local poultry farms and poultry slaughterhouses. For poultry farms, biosecurity measures are mandatory and annual farm licenses are only issued if the farm can demonstrate adequate biosecurity measures. These measures include:

- a) Complete perimeter fencing for all poultry farms and slaughterhouses;
- b) Bird proofing for all poultry houses;
- c) Disinfection facilities for personnel and vehicles;
- d) Restriction of access to premises including restriction of casual visitors.

#### Biosegregation

In addition to biosecurity, AVA has also encouraged

local poultry farms to adopt biosegregation measures. AVA defines biosegregation measures as measures to achieve minimal or no contact between poultry farms to minimise the risk of spread of disease. These measures include:

- a) Separation of egg production and collection areas;
- b) Use of dedicated vehicles for collection of eggs and manure and for delivery of feed, day-old chicks etc;
- c) No sharing of workers or equipment between farms.

The adoption of biosegregation measures by poultry farms has allowed AVA to isolate local farms (4 layer and 2 quail farms) into biosegregated clusters.

#### Surveillance for HPAI

In tandem with enforcement of biosecurity measures, AVA also carries out extensive surveillance for HPAI. These include surveillance on local poultry, imported poultry and eggs, migratory wild birds, pest and urban birds, ornamental birds, birds in wildlife reserves (e.g. Singapore Zoological Gardens and Jurong Birdpark) and birds at reservoirs and parks such as the Botanic Gardens. The results of this surveillance are shown in Table 1. To date no HPAI positive bird has been detected.

Table 1. Number of Birds/Samples Tested for HPAI by AVA

	Number of birds/samples		
	2005	2006	2007
Local poultry	3267	4162	4630
Imported poultry and eggs	5636	6511	6885
Migratory wild birds	296	668	297
Pest and urban birds	1202	2028	1250
Ornamental birds	2279	2408	3460
Wildlife reserves, parks and reservoirs	576	632	849

AVA: Agri-Food and Veterinary Authority; HPAI: highly pathogenic avian influenza

#### Risk-based Vaccination

Vaccination has been shown to be a powerful tool to support eradication programmes in situations in which a stamping-out policy is neither pursuable nor desirable.<sup>17</sup> AVA has implemented a limited risk-based vaccination programme for high-risk species (*Galliformes* and *Anseriformes*) as well as birds kept in open exhibits in the Singapore Zoological Gardens and Jurong Birdpark. Swans and ducks in the Singapore Botanic Gardens have also been vaccinated.

In the Singapore Zoological Gardens, some of the species that were vaccinated include peafowl, Bar-Headed geese,

spotted wood ducks, Egyptian geese, guinea fowl and domestic ducks.<sup>18</sup> The vaccine used is the Nobilis Influenza H5N2, Intervet, an inactivated Avian Influenza Type A H5N2 virus. This vaccine was evaluated and used to vaccinate poultry flocks in Hong Kong.<sup>19</sup>

#### *Improvement of Diagnostic Laboratory Capability*

AVA's Animal and Plant Health Laboratories (APHL) carry out tests for AI. Prior to 2003, culture in embryonated chicken eggs was used as a routine test for AI screening which could take up to 2 weeks to complete. Virus isolation was followed by the Intravenous Pathogenicity Index (IVPI) Test to determine the pathogenicity of the isolate.<sup>20</sup> In addition, AI viruses were also sent to the Veterinary Laboratories Agency (VLA), an OIE Reference Laboratory, in Weybridge, UK, for confirmation. The whole process, from the preliminary diagnosis to confirmation, could take from several weeks to 2.5 months to complete.

To significantly shorten the test-turnover time, APHL decided to adopt molecular techniques to expedite the diagnosis of HPAI. A series of real-time reverse transcriptase polymerase chain reaction (RRT-PCR) assays for the detection of AI type A (matrix gene), AI subtypes H5, H7 and N1 were developed. RRT-PCR offers a much more rapid alternative to virus isolation, with results available within 7 hours. The primers and probes used for the matrix gene were as published by Spackman et al,<sup>21</sup> and the sequences for the primers and probes for H5 and H7 subtypes were unpublished data obtained from Dr Spackman through personal communication. The N1 assay was developed using in-house primer and probe. The assay was validated using 2 HPAI isolates A/Chicken/Hong Kong/220/97 and A/Chicken/Nakorn-Pathom/Thailand/CU-K2/04 (H5N1) and 2 local low pathogenic AI isolates H4N1 and H7N1 from ornamental birds.

APHL has also introduced genetic sequencing and analysis to determine the pathogenicity of AI isolates based on the amino acid sequence at the haemagglutinin gene cleavage site.<sup>20</sup> The primers used for AI subtypes H5 and H7 were as published respectively by Ito et al<sup>22</sup> and Munch et al.<sup>23</sup> This assay method allows classification of the virus into HPAI within 2 days.

The new laboratory capability has significantly reduced the test turnover time from an average of 2 months to 2.5 days.

#### *Public Education*

AVA has embarked on a public education campaign to educate the public to keep pet birds and pet poultry properly caged to avoid contact with wild birds. The AVA web-site has a series of useful frequently asked questions (FAQs) on "bird flu" or Avian Influenza.<sup>24</sup> These range from what is

bird flu, how is it spread, can it be transmitted to humans, etc.

For pet birds, the public has been advised to take precautionary measures such as keeping their birds in a bird-proof enclosure (e.g. cage, hen house or a netted area in their gardens) so that they do not come into contact with wild birds. In addition, owners should not introduce birds of unknown origin to their existing pet birds. Owners or anyone who handles pet birds should also practise good hygiene, such as washing hands thoroughly with soap after handling their pets.<sup>24</sup>

#### *Removal of Backyard Poultry*

In conjunction with AVA's public education campaign on "bird flu", AVA has also taken steps to remove backyard poultry from Pulau Ubin. This is in recognition that backyard poultry are difficult to biosecure or keep caged and the presence of such flocks is a risk factor for HPAI. It has been shown in several Asian countries that once the HPAI virus is entrenched it is extremely difficult to eradicate.<sup>25</sup> The reasons for this are multiple but include high prevalence of backyard flocks, live poultry markets, mixed species farming and legal and illegal wild bird trade.<sup>26</sup>

In addition, AVA has passed legislation that prohibits the keeping of more than 10 pet poultry (in non-commercial premises) and these must be caged. AVA also prohibits the keeping of pet poultry within 1 km of any commercial poultry farms.

### **Emergency Preparedness**

#### *Contingency Plans*

AVA has drawn up its contingency plans to prepare for an outbreak of HPAI in local poultry farms or bird holding premises. In summary these plans cover:

- a) The reporting system for suspect cases, quarantine of suspected premises and outbreak investigation;
- b) Activation and recall of personnel in a suspected outbreak;
- c) The selective culling of biosegregated clusters of poultry farms (in a confirmed outbreak);
- d) Transport and disposal of poultry carcasses;
- e) The roles that other government agencies, such as the Ministry of Health, Singapore Civil Defence Force, Singapore Police Force, play in an outbreak;
- f) Decontamination of personnel, equipment and vehicles; and
- g) Disinfection of premises after de-population of poultry.

In addition, AVA has carried out preparatory measures to stockpile personal protective equipment (PPE), oseltamivir tablets for prophylaxis<sup>27,28</sup> and supplies and equipment for

culling operations. AVA has also drawn up contracts with the private sector to provide services such as disposal, logistics and supply of labour.

#### *Training and Exercises*

AVA has an ongoing training programme to train its officers in biosafety including how to wear PPE, decontamination and mask fitting.

In addition, AVA also carries out exercises (code named *Exercise Gallus*) (Fig. 1) once or twice a year to test its contingency plans. Exercises are carried out in situations that simulate an actual HPAI outbreak. These exercises test AVA's readiness to handle an outbreak in areas such as activation and recall of personnel, outfitting of staff with PPE, logistic support, coordination with other agencies, decontamination procedures and culling of poultry. To date, since February 2004, AVA has carried out 5 exercises.

#### *Emergency Vaccination*

Vaccination has been shown to be a useful tool to prevent and control AI in poultry. The primary goal of vaccination is to prevent or reduce clinical disease from an infectious agent.<sup>29</sup> Other than disease control, vaccination has 2 other important benefits. First, if vaccinated animals become infected, there is reduced virus shedding into the environment.<sup>30,31</sup> This reduction in virus shedding would mean fewer viruses in the environment and would result in more rapid elimination of the virus from the environment.<sup>29</sup> Second, vaccination increases the minimum dose of virus that is required to infect an animal.<sup>32</sup> The increased resistance to infection coupled with reduced virus shedding greatly increases the chance of breaking the infection cycle.<sup>29</sup>

Emergency vaccination was used in Italy to control H7 LPAI in turkey and poultry flocks between 2000 and 2004.<sup>33</sup> Vaccination was also used in the United States to control and eradicate LPAI.

AVA has stockpiled the Nobilis Influenza H5N2, Intervet, inactivated vaccines for emergency vaccination of local poultry farms if the threat of HPAI is imminent. An imminent threat refers to widespread uncontrolled HPAI outbreaks in neighbouring countries' provinces or states in close proximity to Singapore, HPAI detected in wild birds in Singapore and the threat of HPAI infecting humans. AVA will assess the threat before making a decision whether to vaccinate local poultry farms. AVA will also assess other AI vaccines for their effectiveness against H5N1.

#### **Conclusion**

HPAI is a serious poultry disease. In view of the devastating consequence it causes to the poultry industry and the potential for the virus to mutate into a pandemic flu



Fig. 1. Exercise Gallus.

virus, countries all over the world adopt various strategies best suited to their needs and poultry production systems to prevent and control HPAI. A strong veterinary service with adequate technical manpower and financial resources to devise strategies and implement surveillance and control programmes, and a well developed poultry industry with high standards of biosecurity are key success factors in combating HPAI. Some are very successful while others less so. Countries like Malaysia and the UK do not rely on vaccination but adopt import control and biosecurity to keep out HPAI.<sup>34</sup> They have also successfully stamped out occasional incursions of HPAI. Hong Kong has opted for universal vaccination and culling to contain the disease with some degree of success.<sup>19</sup> After stamping out a major outbreak of H7N7 HPAI in 2003, the Netherlands has adopted a preventive, voluntary vaccination programme in the face of the current threat of H5N1.<sup>35</sup> AVA has applied the key elements of disease control principles in developing an appropriate strategy and put in place a series of control measures to prevent the introduction of HPAI. To control any potential outbreak, AVA has also drawn up its contingency plans and taken preparatory measures through simulation exercises. This strategy has been effective in keeping the disease out of Singapore which remains free from HPAI.

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