世界動物衛生組織（OIE） 陸生動物健康手冊（Terrestrial Manual, 2009）

第 2.3.4 章　　禽流感（Avian influenza）

第 2 節　病毒致病性的評估（Assessment of pathogenicity）

台灣動物社會研究會（摘譯）/2012.3.5

The term HPAI relates to the assessment of virulence in chickens and implies the involvement of virulent strains of virus. It is used to describe a disease of fully susceptible chickens with clinical 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, 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. At the First International Symposium on Avian Influenza held in 1981 (6), it was resolved to abandon the term 'fowl plague' and to define HPAI strains on the basis of their ability to produce not less than 75% mortality within 8 days in at least eight susceptible 4- to 8-week-old chickens inoculated by the intramuscular, intravenous or caudal air sac route. However, this definition proved unsatisfactory when applied to the viruses responsible for the widespread outbreaks in chickens occurring in 1983 in Pennsylvania and the surrounding states of the United States of America (USA). The problem was mainly caused by the presence of a virus of demonstrable low pathogenicity in laboratory tests, but which was shown to be fully pathogenic following a single point mutation. Further consideration of a definition to include such 'potentially pathogenic' viruses was undertaken by several international groups.

HPAI 這個詞涉及禽類動物身上的毒株與毒性。用於描述禽鳥感染的疾病，其臨床症狀包括眼鼻流水，呼吸困難，減少食物和飲水的攝取，皮膚發紺、腹瀉等。對蛋雞而言，可能會出現產蛋量減少，或不良雞蛋的數量增加。而高罹病率也通常會伴隨急遽攀升的死亡率。但這些臨床症狀都不能當作診斷病毒致病性的依據[1]。**OIE** 先是於第一屆國際禽流感研討會（**1981**）後，放棄使用「雞瘟（**fowl plague**）」一詞，改以雞隻活體動物試驗（死亡率 **75**％），判定高病原禽流感病毒（**HPAI**）。然而此一定義後來被證實，無法適用 **1983** 年發生於美國賓州的禽流感病毒。問題就在於一個在實驗室中呈現低病原的病毒，在經過簡單的基因突變後，演化成爲高病原。

[1]世界動物衛生組織「陸生動物健康手冊」（OIE Terrestrial Manual, 2009），第 2.3.4 章，（A）引言（introduction）。http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.04_AI.pdf 。

The eventual recommendations made were based on the finding that while there have been numerous isolations of strains of H5 and H7 subtypes of LPAI, all the HPAI strains isolated to date have possessed either the H5 or H7 haemagglutinin. Further information concerning the pathogenicity or potential pathogenicity of H5 and H7 subtypes may be obtained by sequencing the genome, as pathogenicity is 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 gene segments 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 site. Amino acid sequencing of the cleavage sites of H5 and H7 subtype influenza isolates of low virulence for birds should identify viruses that have the capacity, following simple mutation, to become highly pathogenic for poultry. In 1992, the OIE adopted criteria for classifying an AIV as highly pathogenic based on pathogenicity in chickens, growth in cell culture and the amino acid sequence for the connected peptide. The European Union adopted similar criteria in 1992 .

1992 年 **OIE** 採用新的定義：雞隻活體實驗（死亡率），細胞檢測（病變指數）以及氨基酸序列檢定。

The following criteria, which are a modification of the previous OIE procedure, have been adopted by the OIE for classifying an AIV as HPNAI:

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

i)
any influenza virus that is lethal1 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 virus that has an intravenous pathogenicity index (IVPI) greater than 1.2.

The following is the IVPI procedure:
- **Fresh infective allantoic fluid with a HA titre >1/16 (>2 or >log2 4 when expressed as the reciprocal) is diluted 1/10 in sterile isotonic saline.**
- **0.1ml of the diluted virus is injected intravenously into each of ten 4- to 8-week-old SAN 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 death2.)
●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.

For all H5 and H7 viruses of low pathogenicity in chickens, the amino acid sequence of the connecting peptide of the haemagglutinin must be determined. If the sequence is similar to that observed for other highly pathogenic AI isolates, the isolate being tested will be considered to be highly pathogenic.

(see Table 1, which can also be found at: http://www.offlu.net/OFFLU%20Site/Projects/Table%20HPAI%20cleavage%20site%20sequences.pdf).

目前 OIE 規定，必須以符合下述 3 種定義之一，做爲判斷高病原禽流感（HPAI）的準據[2]：

1. 活體動物實驗：以 10 倍稀釋病毒液 0.2ml，靜脈接種 8 隻 4-8 週齡、可感受性（susceptible,無特定病原）雞隻，若 10 天內 6 隻以上雞隻死亡（死亡率 75%），則爲高病原禽流感病毒（HPAI）。

   或（or）

2. 靜脈病變指數（IVPI）檢測：以 0.1ml 毒液靜脈接種，10 隻 4 至 8 週齡雞隻（應儘量使用 SPF 無特定病原雞）。在 10 天內，每 24 小時檢測一次。以呼吸困難（respiratory involvement）、精神沈鬱(depression)、皮膚或雞冠發紺（cyanosis）、頭部或臉部水腫（ oedema）、下痢（diarrhea）及緊張不安（nervous signs）等臨床症狀之一（1 分）或以上（2 分），或死亡（3 分），計算其平均分數。指數爲 3 表示，所有雞隻於 24 小時內死亡。指數爲 0，表示 10 天觀察期間，沒有任何雞隻出現任何臨床症狀。IVPI 指數超過 1.2，即可判定爲高病原禽流感。

3. 所有在雞隻身上表現爲低病原的 H5 或 H7 亞型流感（即其 IVPI 指數小於 1.2，死亡率低於 75% ），仍須進一步檢定其基因序列。如與任何已知其他高病原病毒之基因序列"類似"[3]，則應判定爲高病原病毒。（參見表一）

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[2] 世界動物衛生組織「陸生動物健康手冊」（OIE Terrestrial Manual, 2009），第 2.3.4 章，（B）診斷計技術（Diagnostic Techniques）。http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.04_AI.pdf 。

[3] 已知 H5 及 H7 禽流感病毒基因序列表（OIE 陸生動物健康手冊，2009）。
http://www.offlu.net/OFFLU%20Site/Projects/Table%20HPAI%20cleavage%20site%20sequences.pdf

禽流感病毒致病性高低之判定

(一)OIE 判定標準　　**台灣動物社會研究會/2012.3.6**

	OIE 定義	條件	結果	備註	說明
實驗室	活體動物實驗	**8 隻 4-8 週齡，無特定病原（SPF）雞隻**	**10 天內 6 隻以上雞隻死亡（死亡率 75%），即可判定為高病原禽流感。**	**1992 以前使用的定義**	因田間（蛋雞場或肉雞場）環境條件與病毒特性不一，其臨床症狀不能作為判定病毒致病性的依據。
	靜脈病變指數（**IVPI**）	**10 隻 4～8 週齡，無特定病原（SPF）雞隻**	臨床症狀之一或死亡。指數超過 **1.2**，即可判定為高病原禽流感。		
	檢定基因序列。	病毒分子檢定	縱使其 **IVPI** 指數小於 **1.2**，動物實驗死亡率低於 **75%** ，如基因序列與任何已知其他高病原病毒"類似"（含多個鹼性氨基酸），則應判定為高病原病毒。	因 **1983** 年美國賓州（動物實驗結果）為低病原之病毒，轉變為高病原案例，**1992** 以後 **OIE** 更改定義。澳洲、歐盟等均比照之。	

（二） 台灣自創的判定標準

	判定標準	條件	結果	備註	說明
田間（臨床症狀）	蛋雞場或肉雞場雞隻死亡率（或產蛋率）	以平均每日死亡率約 **0.05**％為基礎。	高病原病毒因此可以解釋為「低病原」。	獨創一格，欺騙社會，矇混國際。	過往農委會防檢局網站資料、學者專家文章，及 **OIE** 文件等，均無此一說法。