

AN OVERVIEW OF HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N1) OUTBREAK CASES IN KELANTAN, WEST MALAYSIA IN YEAR 2017

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ABSTRACT. Malaysia has experienced four waves of H5N1 outbreak but no human deaths were recorded which is in 2004, 2006, 2007 and latest outbreak in 2017 at Kelantan. The objective of this paper is to collect, analyse and summarise the data of HPAI cases from the outbreak from 1 March to 22 June 2017. A total of 1,634 cases, 8,544 samples were received at the Registration Unit and Virology Section, Regional Veterinary Laboratory in Kota Bharu (RVLKB) for diagnosis and surveillance of H5N1 during this period. The samples received were pooled organs and intestines from post-mortem (41 cases) and cloaca swabs in tryptose phosphate buffer from surveillance (1,593 cases), which were sent to RVL, Kota Bharu and Veterinary Research Institute, Ipoh, Perak (VRI). They were processed and diagnosed using quantitative real-time reverse transcriptase PCR (RT qPCR) technique to detect and subtyping of the virus. Total positive cases for H5N1 were 53 out of 1,634 with 18 diagnostic cases and 35 surveillance cases. All positive cases during outbreak were detected from 43 locations from six districts of Kota Bharu, Bachok, Pasir Puteh, Pasir Mas, Tumpat and Tanah Merah, with 19 locations in Kota Bharu which is the highest affected by H5N1, seven locations in Bachok, four in Pasir Putih, six in Pasir

Mas, six in Tumpat and one in Tanah Merah. Many factors could have led to the acute spread of the virus between the districts like chicken smuggling, legal and illegal poultry trade, migration of infected wild birds and others. Understanding the source of outbreak and how it spread is important to control, eradicate and prevent the spread of the disease as it is zoonotic and infects human.

Keywords: H5N1, outbreak, village chickens, Malaysia, Kelantan

INTRODUCTION

Highly pathogenic (HP) avian influenza (AI) (HPAI) is an extremely contagious, multiorgan systemic disease of poultry leading to high mortality, and caused by some H5 and H7 subtypes of type A influenza virus, family Orthomyxoviridae (Swayne *et al.*, 2000). However, most AI virus strains are mildly pathogenic (MP). AI virus strains produce either subclinical infections or respiratory and/or reproductive diseases in a variety of domestic and wild bird species (Swayne *et al.*, 2000). HPAI is a List A disease of the OIE, while MP AI is neither a List A nor List B disease. HPAI viruses do not have a recognised wild bird reservoir, but can occasionally be isolated from wild

birds during outbreaks in domestic poultry (Swayne *et al.*, 2000). HPAI viruses have been documented to arise from MPAI viruses through mutations in the haemagglutinin (HA) surface protein (Swayne *et al.*, 2000). Prevention of exposure to the virus and control/eradication method such as movement control, stamping out and disinfection are the accepted methods for dealing with HPAI (Swayne *et al.*, 2000). The components of a strategy to deal with MPAI or HPAI include surveillance and diagnosis, biosecurity, education, quarantine and depopulation. (Swayne *et al.*, 2000)

History

In 2004, Malaysia first reported an H5N1 outbreak in Kelantan but no human deaths were recorded. Second outbreak was reported in 2006 at Wilayah Persekutuan, Perak and Penang state. A third H5N1 outbreak was reported in Selangor in 2007. That was the last time Malaysia recorded a H5N1 avian influenza outbreak.

The chronology of the outbreak in 2017 started when a private veterinarian from Kota Bharu, Kelantan sent a poultry carcass from Kampung Pulau Tebu, Tunjung Kota Bharu to the RVLKB for routine post-mortem with a history of more than 50% mortality rate on 28 February 2017. Upon post-mortem inspection, the carcass presented with typical AI symptoms such as bluish or purplish discoloration of head, swollen face and haemorrhagic legs with generalised haemorrhagic organ lesions. The laboratory finding was positive for AI H5N1 virus. Sequencing showed HPAI virus HA Clade 2.3.2.1c, which is closely related to

a Vietnam strain. The cases were reported to the Department of Veterinary Service Headquarters in Putrajaya and Kelantan State Veterinary Department took immediate action to eradicate and control the spread of the disease. On 15 March 2017, the Kelantan Government declared the H5N1 epidemic as a state disaster under the MKN 20 (National Security Council) Order after it spread from Kota Bharu to other districts, such as Pasir Mas, Bachok and Pasir Putih. As a consequence, about 56,961 poultry and 17,531 eggs were disposed, involving 1,243 premises.

The objective of this paper is to collect, analyse and summarise the data and cases of HPAI outbreak from 1 March to 22 June 2017, so as to collate information for future control programmes related to HPAI in poultry.

MATERIALS AND METHOD

The data is taken from the laboratory records for HPAI cases. A total of 1,634 cases and 8,544 samples were received at the Registration Unit and Virology Section, RVLKB for diagnosis and surveillance of HPAI starting from 1 March to 22 June 2017. The species involved were village chickens, organic village chickens, ducks, pet birds, bantam chickens, geese, pet birds, other chickens, quails, broiler chickens, ducks, turkeys, swiftlets and cats. Types of samples were pooled organs (trachea, lung, liver, spleen, kidney, heart) and intestine from post-mortem (diagnostic) and cloaca swabs in tryptose phosphate buffer (surveillance). The samples were sent to RVLKB and immediately packed to be sent to VRI on the

same day. During the first two months of outbreak from March to April, the samples were processed and diagnosed in VRI using quantitative real-time reverse transcriptase PCR (RT qPCR) technique to detect the virus and subtyping. However, from May to June, RVLKB manage to do the diagnostic process in its laboratory using same PCR technique except for subtyping. Briefly, the viruses RNA were extracted using High Pure Viral RNA Kit (Roche, Germany), according to the manufacturer's instructions. Reverse transcription was performed by using specific primers: Forward primer (NP1200) 5'-CAG RTA CTg ggC hat AAg RAC-3'; Reverse primer (NP 1529) 5'-Gca TTg TCT CCg AAg AAA TAA g-3' and SensiFAST™ SYBR No-ROX One-Step Kit (Bioline, UK) according to the manufacturer's instructions. The prepared samples were run in the qPCR machine (Biorad, Singapore). The reaction was carried out for 10 min at 45°C (activation) followed by 2 min at 95 °C (initial denaturation), with a subsequent 34 cycles of amplification by denaturation (95 °C for 8 s), annealing (55 °C for 10s) , and extension steps (72 °C for 8 s) and the result will be read based on graph or CT value.

RESULTS AND DISCUSSION

All 1,634 cases were divided into two groups which are diagnostic (41 cases, 87 samples) and surveillance (1593 cases, 8457 samples). From that, 53 cases (diagnostic: 18cases, 42 samples; surveillance: 35 cases, 205 samples) were positive H5N1 (Figure 1) which is 3% in percentage, and based on species there were 66% village chickens, 5% organic village chickens, 13% mix poultry (village

chickens, ducks, pet birds, bantam chickens, and geese), 2% pet birds, 2% other chickens, 2% quails, 2% broiler chickens, 2% ducks and 4% turkeys (Figure 2). Other remaining cases were negative to AI virus. The percentages show that the infection mostly affected village chickens. However, besides village chickens, other poultry species also affected but in low percentage. According to Alders (2014) who stated that village poultry can include a wide range of birds including indigenous and crossbred breeds of chickens, quail, mallard and Muscovy ducks, pigeons, guinea fowl, geese, and turkeys. Of these birds, chickens, quail, guinea fowl, and turkeys are highly susceptible to HPAI.

Overall, there were 1,253 cases of village chickens, organic chickens 7 cases, cockfighting chickens 4 cases, broiler chickens 16 cases, bantam chickens 14 cases, turkeys 3 cases, jungle fowls 8 cases, other chickens 14 cases, ducks 92 cases, pet birds 42 cases, geese 6 cases, swiftlets 8 cases, mix poultry 152 cases and cat 1 case (Figure 3). In percentage, 77% village chickens, organic chickens 0.42%, cockfighting chickens 0.2 %, broiler chickens 0.97%, bantam chickens 0.85%, turkeys 0.18%, jungle fowls 0.49%, other chickens 0.9%, ducks 5.6%, pet birds 2.6%, geese 0.37%, swiftlets 0.49%, mix poultry 9.25% and cat 0.06%. (Figure 4). The percentages show that the cases mostly from village chickens due to high population compared to other bird species in Kelantan.

All positive cases during outbreak were detected in 43 locations from 6 districts, which are Kota Bharu, Bachok, Pasir Puteh, Pasir Mas, Tumpat and Tanah Merah. Meanwhile, positive diagnostic cases were detected in 15 locations, and positive

Table 1. The positive cases and its locations

NO	DISTRICT	LOCATION	NO. OF POSITIVE CASES		
			DIAG	SURV	
1	Kota Bharu	Kg. Pulau Tebu	4	0	
2		Kg Cabang Tiga, Pendek	1	1	
3		Kg Gertak Lembu	0	2	
4		Kedai Mulong	1	3	
5		Kg Aur Duri	0	1	
6		Kg Surau Kota	0	1	
7		Kg Machang Bobok	0	1	
8		Kg Serian	1	0	
9		Kg Jaya	0	1	
10		Kg Kenali	0	1	
11		Kg Padang Mengkali	0	1	
12		Kg Kor	1	0	
13		Kg Parit Cina	1	0	
14		Kg Padang Layang	0	1	
15		Berek 12	0	1	
16		Kg Dusun Raja	1	0	
17		Kg Pauh Butut	0	1	
18		Kg Jelutong	1	0	
19		Kg Pulau Raja	0	1	
20	Bachok	Kg Dusun Durian	0	1	
21		Kg Aman	0	1	
22		Kg Baru Beris Lalang	0	1	
23		Kg Kandis	0	1	
24		Kg Gong Wayang	0	1	
25		Kg Jln Mujur	0	1	
26		Kg Baru Gajah Mati	0	1	
27		Kg Bukit Merbau	0	3	
28		Pasir Putih	Kg Padang Pak Omar	0	1
29			Kg Tualang Rrendah	0	1
30	Kg Selising		0	1	
31	Pasir Mas	Kg Bechah Semak	1	0	
32		Kg Tempoyak	1	0	
33		Kg. Kedondong	1	0	
34		Kg Sg Tendong	1	0	
35		Kg. Repek	0	1	
36		Kg. Kubang Bemban	0	1	
37		Kg Garong	0	1	
38	Tumpat	Kg Cherang Melintang	1	0	
39		Kg Kebakat	0	1	
40		Kg Paloh Dalam	1	0	
41		Kg Belukar	1	0	
42		Kg Kedemit	0	1	
43	Tanah Merah	Kg Paloh Bukit Panau	0	1	

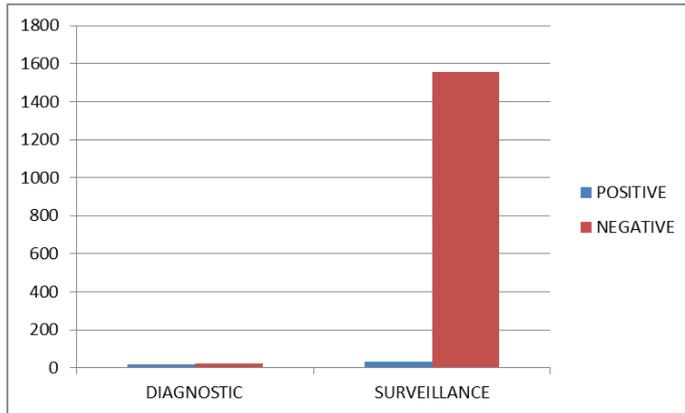


Figure 1. No of positive and negative cases based on diagnostic and surveillance categories

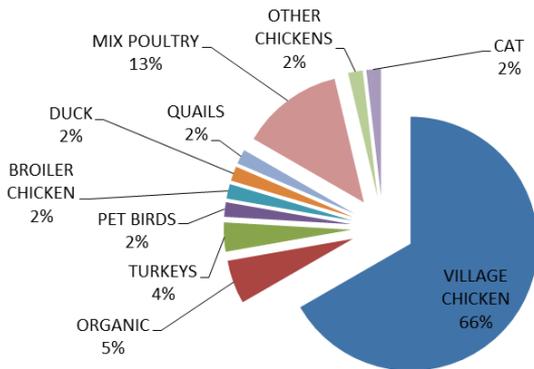


Figure 2. Percentage of positive cases based on species

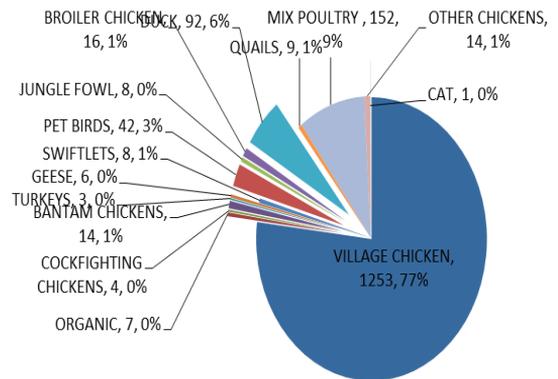


Figure 3. No of cases based on species

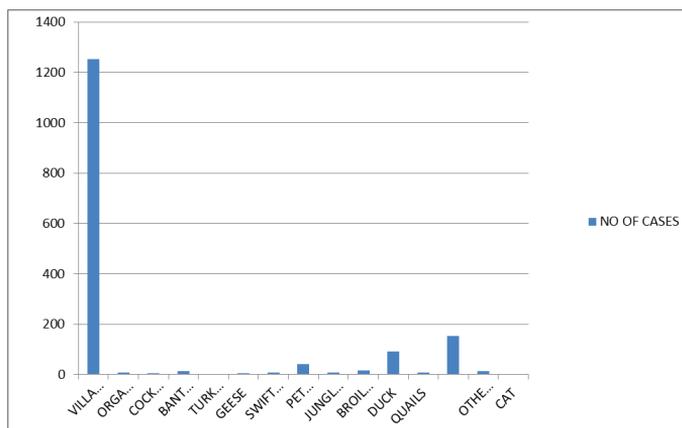


Figure 4. Percentage of cases based on species

surveillance cases in 30 locations. Table 1 shows the positive cases and its locations. Yellow background colors show the positive diagnostic cases.

From the Table 1, there were about 19 location in Kota Bharu which is the highest affected by H5N1 compared to other districts which were recorded, 7 location in Bachok, 4 in Pasir Putih, 6 in Pasir Mas, 6 in Tumpat and 1 location in Tanah Merah.

The disease acutely spread interdistrict starting from Kota Bharu, Pasir Mas, Tumpat, then Bachok, Pasir Putih and finally Tanah Merah. How does the H5N1 virus spread? It spreads mostly through the trade of infected poultry and, to some extent, following the migration of infected wild birds (Springer Nature, 2007). The virus also can be spread by contact between infected and healthy birds, though can also be spread indirectly through contaminated equipment (WHO 2014). Infected birds shed the viruses in their feces, which can drop onto either land or water, where other birds can ingest them via the mouth or nasal passage. Poultry flocks are worst affected, transmitting the virus easily in crowded conditions. The live poultry trade appears to be the main factor in the spread of H5N1 in Asia and Africa (Springer Nature, 2007). By contrast, wild bird migration seems to have contributed to the spread of the virus from Asia to Iran and Europe, although this is still a matter of lively debate (Springer Nature, 2007).

The possible cause of the outbreak is through cockfighting activities involving roosters from neighboring country, which is similar to the last outbreak occurred in 2004 (Nandini B., 2017). Other cause is illegal supplies of village chicken or bird to

the country from the border area (Nandini B., 2017). According to Alders (2014), five man-made ecosystems have contributed to modern avian influenza virus ecology: integrated indoor commercial poultry, range-raised commercial poultry, live poultry markets, backyard and hobby flocks, and bird collection and trading systems including cockfighting. Indoor commercial poultry has had the largest impact on the spread of HPAI, with the increase in HPAI outbreaks largely the result of increased commercial production since the 1990s.

The other possible cause could be the wild birds which are suspected of transmitting this virus to poultry (Keawcharoen *et al.*, 2011). Keawcharoen (2011) studied that transmission efficiency among poultry flocks was 1.7 times higher in regions with infected wild birds. Wild birds and poultry are associated with increased spread among poultry flocks.

The Kelantan Veterinary State Department with cooperation of Veterinary Headquarters and other department/authorities has taken steps to control and eradicate the disease from spread like movement control inside the country, quarantine, screening, disinfection, surveillance outside containment and/or protection zone, stamping out, vaccination prohibited and no treatment of affected animals. With the immediate action taken, Malaysia has been declared free from HPAI H5N1 on July 1, 2017 announced by Veterinary Services Department Director General according to no new occurrences of the disease after 90 days from last disinfection procedure on April 1. The 90 day period is a condition laid down by the

World Animal Health Organization (OIE) in addressing this disease (Bernama, 2017).

CONCLUSION

The positive cases are three percent from total cases, and the percentage is higher in village chickens than other species due to high population of village chickens compared to other species in Kelantan. The infection fast spread to other locations could be caused by many factors such as smuggle chickens out of affected areas, the trade of infected poultry, the migration of infected wild birds and many other factors as mention in the discussion. Understanding the source of outbreak and how it spread is important to control, eradicate and prevent the spread of the disease as it is zoonotic and infect human. Luckily there has been no human H5N1 case detected during the outbreak.

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