

Epidemic Outbreaks, Diagnostics and Control Measures of the H5N1 Highly Pathogenic Avian Influenza (HPAI) in Kingdom of Saudi Arabia, 2007 – 08

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ABSTRACT

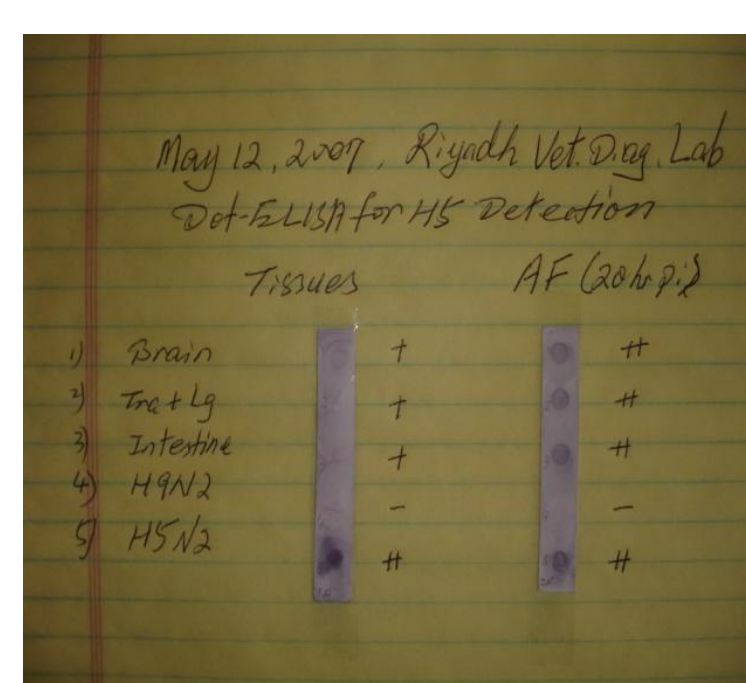
The first outbreak of H5N1 highly pathogenic avian influenza (HPAI) occurred in two "back yard" flocks of Houbara bustards and falcons in Kingdom of Saudi Arabia (KSA) in Feb 2007, and subsequent outbreaks continuously occurred in "back yard" poultry of native chickens ostriches, and turkeys till the end of 2007. Starting November 2007 till January 2008, the H5N1 HPAI outbreaks occurred in 19 commercial poultry premises including 2 broiler breeder farms, 1 layer breeder farm, 1 ostrich farm, and 15 commercial layer farms with approximately 4.75 millions of birds affected. Laboratory diagnosis of all H5N1 positive cases was conducted at the Central Veterinary Diagnostic Laboratory (CVDL) in Riyadh. Combination diagnostic tests were conducted to make most effective and accurate laboratory diagnosis, which include rapid antigen-capture test and real-time RT-PCR assay on clinical and field specimens first, meanwhile virus isolation in specific-pathogen-free (SPF) embryonating chicken eggs followed by HA and HI tests, and then rapid antigen-capture and RT-PCR tests on HA positive allantoic fluid samples. A complete laboratory diagnosis on most HPAI cases was made within 24-48 hrs at the CVDL. Saudi Arabian government officials made immediate decisions of depopulation of all H5N1 affected and non-affected flocks within a 5 km radius area and application of quarantine zone to prevent the virus from spreading to other areas. Other control measures such as closure of live bird markets and intensive surveillance tests on all poultry species within quarantine zones were in place during the outbreaks. As a result, the HPAI outbreaks were quickly controlled and no more positive cases being detected so far after the last H5N1 case was diagnosed on January 29, 2008. KSA declared free of HPAI status by April 30, 2008 according recommendations of World Animal Health Organization.

MATERIALS AND METHODS

Epidemiological Investigation. Commercial and "back yard" poultry flocks of all avian species were investigated and reported to the Ministry of Agriculture if any clinical signs or suspicious of HPAI infection. Dead or sick birds or clinical samples were collected and submitted to the Central Veterinary Laboratory (CVDL) in Riyadh.

Specimens. Specimens collected for HPAI diagnosis include tissues of trachea, lung, brain and intestine from dead birds or sacrificed sick birds, tracheal and cloacal swabs from live birds, and sometimes environmental swabs from affected poultry houses. Tissue specimens were grinded and diluted with sterile PBS at 1:5 (wt/vol) dilutions and then centrifuged at 1000 rpm for 10 min. The supernatant was collected for AIV diagnostic tests by rRT-PCR and rapid antigen-capture tests. The supernatant was treated with multi-antibiotics mixture for virus isolation in embryonating chicken eggs (ECE).

Diagnostic tests. AIV diagnostic tests conducted at CVDL in KSA include the BinaxNOW® Influenza A test (3), Dot-ELISA for AIV group and H5 subtype (4), two steps of rRT-PCR test for H5 and N1 subtype (2) and virus isolation using specific-pathogen-free (SPF) embryonating chicken eggs (ECE) (Venky's Ltd, Pune, India) following published procedure (6). A combination of these diagnostic tests provided most effective and accurate laboratory diagnosis of HPAI at CVDL. Clinical and field specimens were tested first by rapid antigen-capture and real-time RT-PCR assays and if positive or suspicious, the specimens were inoculated into ECE for virus isolation. Allantoic fluids (AF) harvested from the inoculated ECE were tested for the presence of AIV by HA and HI (to H5 subtype) tests, the BinaxNOW® Influenza A test and Dot-ELISA for AIV group and H5 subtype, and rRT-PCR for H5N1 virus.



Dot-ELISA for H5 subtype

Trachea, lung, brain and intestine from the dead bird were tested positive for H5 subtype of AIV by the Dot-ELISA using H5 subtype-specific monoclonal antibody.

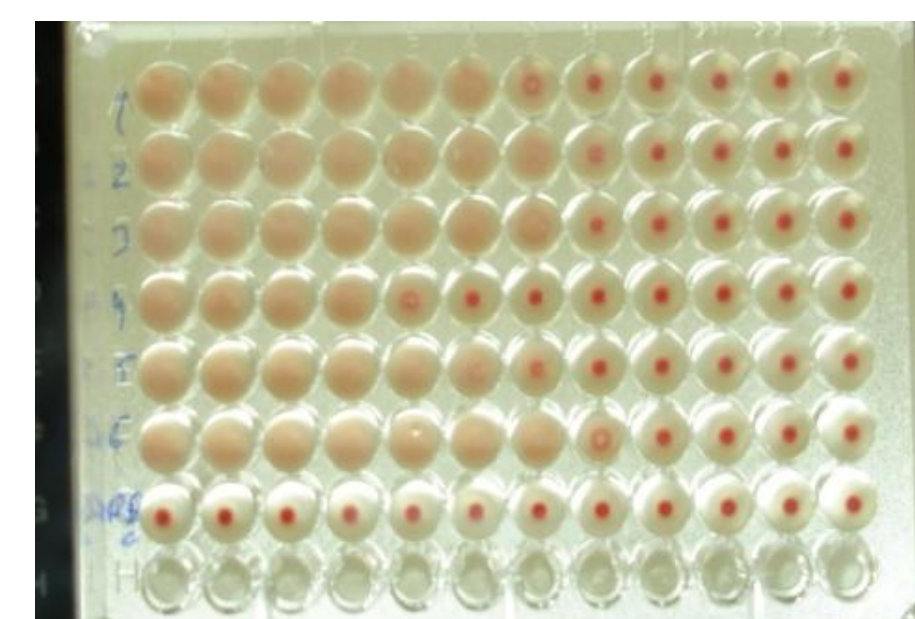


BinaxNOW® Influenza A & B test

AIV tissue specimens were tested positive for Influenza A virus by the BinaxNOW® Influenza A & B test.



H5N1 killed embryos 16 pi with severe hemorrhagic lesions



HA titers 1:16 – 1:128 AF from dead embryos 16 hrs pi

RESULTS

From Feb till December 2007, H5N1 outbreaks continuously occurred in 24 "back yard" poultry flocks and wild bird cases with different species including Houbara bustards, falcons, wild bird, native chickens, ostriches, turkeys, ducks, peacocks (Table 1).

Table 1. The HPAI H5N1 positive cases/outbreaks occurred in native chickens and other poultry species of "Backyard" flocks in family rest houses in Kingdom of Saudi Arabia in 2007

Serial No.	Date of outbreak (HH5N1 diagnosed)	Poultry Species
1	2/09/07	Houbara bustard ^A
2	2/09/07	Falcons ^B
3	3/12/07	Ostrich (Hobby bird)
4	4/??/07	Wild birds
5	5/09/07	Chicken ^C
6	6/14/07	Chicken
7	6/27/07	Chicken
8	10/24/07	Turkey
9	10/29/07	Chicken
10	11/02/07	Turkey
11	11/09/07	Chicken
12	11/15/07	Chicken, Duck
13	11/17/07	Chicken
14	11/17/07	Duck
15	11/17/09	Chicken
16	11/19/07	Cock
17	11/19/07	Chicken
18	11/20/07	Chicken
19	11/20/07	Chicken
20	11/20/07	Chicken
21	11/25/07	Chicken
22	11/27/07	Chicken
23	11/29/07	Chicken, duck
24	12/03/07	Turkey
25	12/03/07	Peacock

Massive epidemic outbreaks occurred in 19 commercial poultry premises from Nov 2007 to Jan 2008 (Table 2)

Table 2. The H5N1 HPAI outbreaks occurred in commercial poultry farms in Kingdom of Saudi Arabia in 2007 – 2008

Serial No.	Date of outbreak (HPAI diagnosed)	Type of production
1	11/12/07	Broiler Breeder
2	11/14/07	Commercial Layer
3	11/15/07	Commercial Layer
4	11/15/07	Commercial Layer
5	11/17/07	Commercial Layer
6	11/17/07	Commercial Layer
7	11/18/07	Layer Breeder
8	11/21/07	Commercial Layer
9	11/26/07	Commercial Layer
10	11/26/07	Commercial Layer
11	11/27/07	Commercial Layer
12	11/28/07	Commercial Layer
13	11/30/07	Commercial Layer
14	12/03/07	Commercial Layer
15	12/09/07	Commercial Layer
16	12/10/07	Commercial Layer
17	12/12/07	Ostrich
18	12/16/07	Broiler Breeder
19	01/29/08	Commercial Layer

The HPAI outbreak in 19 commercial poultry premises were all located around Riyadh area and adjacent north, south and east to Riyadh of four governorate regions named Al Kharj, Durma, Mezahmyia and Thadek. The 19 commercial poultry premises include 2 broiler breeder farms, 1 layer eeder farm, 1 ostrich farm, and 15 commercial layer farms as shoed below in Table 3. A total number of 4,749,598 birds in the 19 affected poultry farms and 867,748 birds in 8 non-affected farm within the 5 km radius infected zones were all depopulated.

Table 3. The H5N1 HPAI outbreaks in commercial poultry farms and "stamping out" farms within infection zone during outbreaks in Kingdom of Saudi Arabia, 2007 - 2008

Governorate	Type of production	HPAI affected farm		Non-affected farm	
		No. of farm	No. of bird	No. of farm	No. of bird
Al Kharj	Broiler breeder	2	469,874	1	96,000
	Layer	12	3,969,264	4	420,850
	Ostrich	1	13,460	0	-
	Broiler	0	-	1	210,000
Durma	Layer	1	43,000	1	100,898
	Layer breeder	1	35,000	0	-
	Broiler breeder	0	-	1	40,000
Mezahmyia	Layer	1	75,000	0	-
Thadek	Layer	1	144,000	0	-
Total		19	4,749,598	8	867,748

Epidemic patters of the disease outbreaks or onsets, illustrated in figure 1, revealed that the outbreaks were initially from backyard birds and finally spread to commercial poultry, and caused a peak of outbreaks in both backyard and commercial poultry in November 2007.

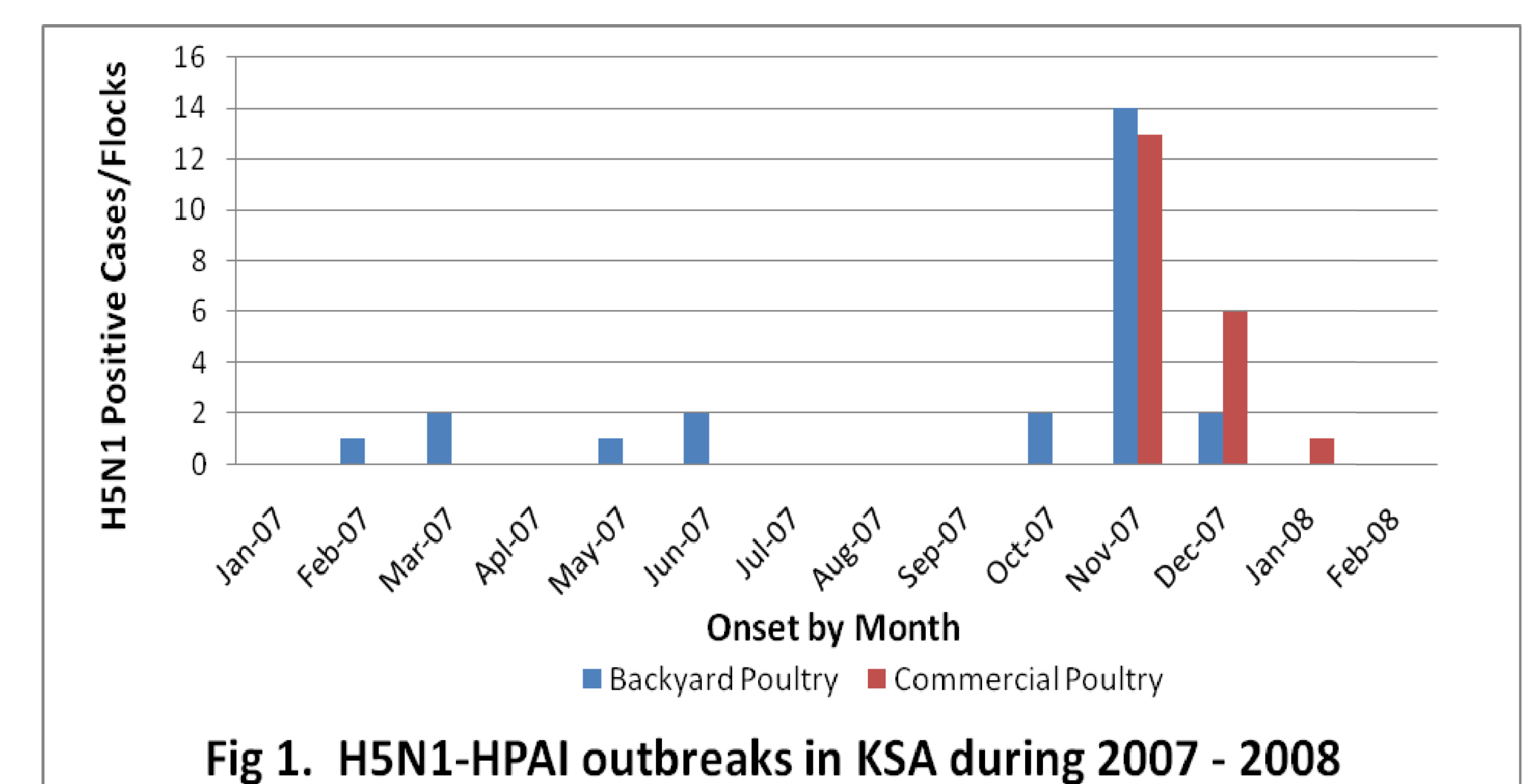


Fig 1. H5N1-HPAI outbreaks in KSA during 2007 - 2008

During the HPAI outbreaks, a total number of 1925 clinical and field samples from 385 submissions were tested at the CVDL in Riyadh. Two steps of rRT-PCR were conducted on pooled samples for each of the 385 submissions, 47 out of the 385 submissions were tested H5N1 positive by rRT-PCR, and 43 of 47 were confirmed H5N1 positive cases by virus isolation in ECE and then followed by HA/HI, the BinaxNOW® Influenza A test or Dot-ELISA, and rRT-PCR test on AF specimens. The 43 H5N1 cases include 23 backyard poultry flocks, 1 wild bird case, and 19 commercial premises. Each of the 43 cases was confirmed being HPAI H5N1 positives by OIE/FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza in Padova, Italy.

Acknowledgement

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