Epi-zone approach for HPAI H5N1 surveillance and control



- 1. Identification of HPAI H5N1 epi-zones in Asia
- 2. Epi-zone approach for surveillance and control
- 3. Laboratory requirement for effective surveillance

Ken Inui, laboratory expert, FAO ECTAD VN

Blood type: B, Aquarius, Image color: White Ken.inui@fao.org

What is "Epi-zone"?

- Regions that shares closely related viruses and common epidemiological features, risk factors
- Determinants
 - Virus character
 - Geographical location
 - Movement of birds
 - The others





0.005



H5N1 outbreaks in Asia 2011-12



*Officially reported cases only (Empres-i Asia)



H5N1 outbreaks in Asia 2011-12 HA clade distribution by country

Clade 2.3.2.1 virus detected in 12 of 14 countries Clade 2.3.4 still circulating in South Asia

			East	t			Sou	th I	East			Sou	uth	
HA Clade	Japan	Korea	China	Mongolia	Hong Kong	Viet Nam	Laos	Cambodia	Indonesia	Myanmar	Bangladesh	India	Nepal	Bhutan
7.2			Χ											
1.1						X		X						
2.1.3.2									Χ					
2.2											Χ			
2.3.4										Χ	Χ		Χ	
2.3.2.1	Χ	Χ	Χ	Х	Χ	Х	Х			Χ	Χ	Χ	Χ	Χ



5 clusters of clade 2.3.2.1 virus

			ĺ	East	t			Sc	outh	n Ea	st			Sou	uth	
HA Clade		Japan	Korea	China	Mongolia	Hong Kong	Viet Nam (North)	Viet Nam (South)	Laos	Cambodia	Indonesia	Myanmar	Bangladesh	India	Nepal	Bhutan
7.2				Χ												
1.1								X		Χ						
2.1.3.2											Χ					
2.2																
2.3.4												X	Χ		Χ	
	Α			Χ			X									
	В			Χ		Χ	X									
2.3.2.1	С					X	X									
	D	Χ	Χ		Χ										Χ	
	Ε											X	Χ	Χ	Χ	Χ

Epi-zone 5 Indonesia

				East					South	n East				So	uth		
HA Clade		Japan	Korea	China	Mongolia	Hong Kong	Viet Nam (North)	Viet Nam (South)	Laos	Cambodia	Indonesia	Myanmar	Bangladesh	India	Nepal	Bhutan	
7.2				х													
1.1								х		х							
2.1.3.2											х						
2.3.4												х	х		х		
	Α			х			х		х								
	В			х		х	х										
2.3.2.1	С					х	х										
	D	х	х		х										х		
	E											х	х	х	х	х	



Clade 2.1.3 viruses in Indonesia



Epi-zone 4 Cambodia, Vietnam (South)

				East					South	n East				So	uth	
HA Clade		Japan	Korea	China	Mongolia	Hong Kong	Viet Nam (North)	Viet Nam (South)	Laos	Cambodia	Indonesia	Myanmar	Bangladesh	India	Nepal	Bhutan
7.2				х												
1.1								х		х						
2.1.3.2											х					
2.3.4												х	х		х	
	Α			х			х		х							
	В			х		х	х									
2.3.2.1	С					х	х									
	D	x	х		х										х	
	E											х	х	х	х	х



Clade 1 H5N1 viruses 1996-2012

					Y	ear					
Countries	1996 -2002	20 03	20 04	20 05	20 06	20 07	20 08	20 09	20 10	20 11	20 12
China											
Vietnam (North)											
Laos											
Vietnam (South)											
Cambodia											
Thailand											

- Clade 1 H5N1 virus was prevalent in south China early 2000s.
- It was introduced to Indochina in 2003, and has caused a severe epidemic during 2003-05 in Vietnam, Laos, Cambodia, Thailand.
- Now (2009-2012) it is confined to lower Mekong delta region i.e. south Vietnam and Cambodia.

Clade 1 H5N1 viruses in Vietnam and Cambodia



Epi-zone 1: Japan, Korea, (North China), (Siberia)Epi-zone 8: Nepal, Mongolia, (West China), (Siberia)Epi-zone 9: Mongolia, (Siberia)

				East					South	n East				So	uth	
HA Clade		Japan	Korea	China	Mongolia	Hong Kong	Viet Nam (North)	Viet Nam (South)	Laos	Cambodia	Indonesia	Myanmar	Bangladesh	India	Nepal	Bhutan
7.2				х												
1.1								х		х						
2.1.3.2											х					
2.3.4												х	х		х	
	Α			х			х		х							
	В			х		х	х									
2.3.2.1	С					х	х									
	D	х	х		х										х	
	E											Х	х	х	Х	х

Activity of viruses in these epi-zones seems to be low in 2012



Epi-zone 3

South China, Hong Kong, Vietnam (North), Laos

				East					South	n East				So	uth		
HA Clade		Japan	Korea	China	Mongolia	Hong Kong	Viet Nam (North)	Viet Nam (South)	Laos	Cambodia	Indonesia	Myanmar	Bangladesh	India	Nepal	Bhutan	
7.2				х													
1.1								х		х							
2.1.3.2											х						
2.3.4												х	х		х		
	Α			х			х		х								
	В			х		х	х										
2.3.2.1	С					х	х										
	D	х	х		х										х		
	E											х	х	х	х	х	





0.005

Clade 2.3.2.1.C = A/HK/6841/2010



Epi-zone 2 China

All 10 HA clade are found in this epi-zone during 1996-2012

Epi-center of H5N1

China																			
								H	IA c	lade	s								
Year	0	4	2.4	2.2		2	.3		24	2 5	2	4	E	6	7	0	0	 +	Total
		<u> </u>	2.1	2.2	1	2	3	4	2.4	2.5	3	4	5	0	1	0	9	Oui	
96-99	9																		9
2000	8												1						9
2001	17										8		1				1	1	28
2002	12	6	1						2			4	2	1		3	2	8	41
2003	4	9	1		1	1			14	4	1	1	3		1	1	6	4	51
2004	4				3	11	1		9	1			16	3	3		13	3	67
2005				19	12	41	5	34	2	0		1			7		9	1	131
2006				4		3		63		2		5			1		1		79
2007																			0
Total	37	15	2	23	16	56	6	97	27	7	9	11	22	4	12	4	32	17	397



Epi-zone 6 Myanmar, South China

Clade 2-3-4 and 7 were detected in the past when those clade were prevalent in China; epi-zone 2

Probably the route of virus transmission from China to South Asia



Epi-zone 7 Myanmar, Bangladesh, India, Nepal, Bhutan

				East					South	n East				So	uth		The standard and
HA Clade		Japan	Korea	China	Mongolia	Hong Kong	Viet Nam (North)	Viet Nam (South)	Laos	Cambodia	Indonesia	Myanmar	Bangladesh	India	Nepal	Bhutan	
7.2				х													
1.1								х		х							State of the state
2.1.3.2											х						and the second
2.3.4												х	х		х		
	Α			х			х		х								
	В			х		х	х										030
2.3.2.1	С			х		х	х										
	D	х	х		х										х		
	E											х	х	х	х	х	



Clade 2.3.2.1 in Epi-zone 7



Clade 2321 virus in epi-zone 7 forms distinct cluster within 2321, and not directly related with those in epi-zone 3

- Virus of 2011 and 2012 from Bangladesh, India, Nepal, Bhutan are mixed up
- Indicates that frequent virus exchange
- Where is the epi-center?



Clade 2.3.4 in Epi-zone 7



H5N1 Epi-zones in Asia



Epi-zone approach for H5N1 surveillance and control

Vietnam as an example

Epi-zone approach for HPAI surveillance and control

- Objectives of the surveillance
 - Identify the risk factors for HPAI virus introduction and circulation
- Regions in the same epi-zone would have the common risk factors
- Control of HPAI
 - Mitigate the risks for virus circulation
- HPAI surveillance and control should be based on epizone by epi-zone instead of country by country.
 - Need for the cross-border talk

Change of H5N1 HA clade in Vietnam 2003-2012

				No	. of H5 vir	uses in H	A clade		
Voor						2			
fear	0	1	5	1 21	224	7			
				2.5.2	А	В	С	2.5.4	
2003		17	2						
2004		70							
2005	3	73		9				10	
2006		4							
2007		42						103	
2008		7						53	18
2009		19			3			30	
2010		12			22			18	
2011		23			68	6			
2012 (Jan-Jun)		17			32	10			
2010 (July-Oct)		4			17	5	146		

Change of HA Clade in the North Vietnam during 2008-2011 (Oct)



Month	Jan - Dec	Jan - June	July - Dec	Jan - June	July - Dec	Jan - Oct
Year	2008	20)09	20	010	2011

Change of HA Clade in the North Vietnam during 2011(Nov)-2012 (June)



Month	Nov - Dec	Jan - Feb	March - May	June
Year	2011		2012	

Emergence of a new cluster 2.3.2.1.C and its quick and wide spread in July -**August 2012**



H5N1 Virus Circulation and Incursion in the north Vietnam



Frequent incursions of H5N1 viruses into the north Vietnam

The introduced virus spread and circulate locally resulting in the small local epidemic, but eventually dies out.

H5N1 Virus Circulation and Incursion in the south Vietnam



Zonal approach for HPAI H5N1 surveillance and control in Vietnam (personal view)

	North (Epi-zone 3)	South (Epi-zone 4)
Virus in circulation	Changing Currently 2321A, B, and C Old viruses died out	Clade 1 has kept circulating since its first introduction in 2003
Virus incursion	Frequent	Rare
Virus persistence	Duck	Duck
Chance of control / eradication	Yes If new incursion can be stopped	Yes Once eradicated, virus incursion is rare
Surveillance	Hot provinces on the route of virus incursion	Extensive surveillance at low season
Control methods	Stamp out H5 positive flocks at hot provinces Use of DEV-H5 vaccine	Stamp out H5 positive flocks at low season Use of DEV-H5 vaccine?

Possible routes for frequent virus incursions 5 provinces where all A, B, and C were detected

		Clade virus				
No.	Province	2.3.2.1A	2.3.2.1B	2.3.2.1C	Total	
1	Son La		1	2	3	
2	Thai Nguyen	3	2	4	9	
3	Tuyen Quang			3	3	
4	Lang Son	1		3	4	
5	Bac Kan	1	1	2	3	
6	Dien Bien	1			1	
7	Bac Ninh	2	2	8	12	
8	Bac Giang	3			3	
9	Ha Nam	3		3	6	
10	Ha Noi	5			5	
11	Hai Duong	3			3	
12	Hai Phong	8		2	10	
13	Nam Dinh	2		10	12	
14	Ninh Binh	1	1	10	12	
15	Phu Tho			2	2	
16	Quang Ninh	4	1		5	
17	Thanh Hoa	1		1	2	
18	Ha Tinh			2	2	
19	Nghe An	5	2	5	12	
20	Quang Binh			11	11	
21	Quang Nam	1			1	
22	Quang Ngai			3	3	
23	Quang Tri	4		4	8	
	Total	48	10	75	133	



Cross-Border Talk Epi-zone 4: Vietnam and Cambodia

- Harmonization of HPAI surveillance and control
 - Strategy, methods, and implementation
 - Lots of challenges; different system, capacity, budget, etc.



Laboratory requirements for effective surveillance

- Targets
 - Quick (within 24 hours after receipt of samples)
 - Accurate (sensitive and specific)
 - Cheap (low running cost)
 - Easy (to perform, to monitor the performance quality)
 - High throughputs

- Real-time RT-PCR
 - One SOP at all animal health laboratories
 - Use of several sets of primers for one target virus
 QA
- Virus isolation
- Virus genetic characterization capacity

Capacity to update primers and probes for qRT-PCR

- Validate with local isolates
 - Efficiency and sensitivity (Fig 1)
 - May need modification as the virus continues to evolve (Fig 2)
 - Requires panel of RNA/DNA for specificity tests
 - Requires capacity to monitor mutations at primer regions



Fig 1 Efficiency test for H5N1 viruses 2011

DNA sequencing without your own sequencer

• Needs

- Strain differentiation for molecular epidemiology
 - Influenza A (HA1), PRRS (ORF5), CSF (E2), etc.
- Monitoring of mutations at primer positions
 - Influenza A (HA2), PRRS (NSP2)
- Requirements
 - Cheap, accurate, quick , and sustainable



- Prepare PCR products with sequencing primers
 - All sequencing primers are tagged with M13 universal primers, so that one primer can be used for sequencing of any genes of any viruses at a time

220 230 240 GATTTTAAAAGATTGTAGTGTAGG

- Send PCR products to commercial sequencing service
 - Purification and direct sequencing of PCR products with M13 primers
- Receive results within 3-4 days after shipment
- Advantages
 - Good quality sequencing
 - Usually >800bases of >QV20
 - Cheaper than sequencing on your own
 - USD3-6 per reaction including purification, free shipping for >20 reactions
 - No maintenance cost of sequencer

Vaccine efficacy for 2321 viruses

H5N1 vaccines used in Vietnam

- Re-1:
 - clade 0
 - Strain: A/goose/Guangdong/1/1996
 - Used 2006-2010
- **Re-5**:
 - clade 2.3.4
 - Strain: A/duck/Anhui/1/2006
 - Used in 2011-12 mainly in the south
- Re-6:
 - Clade 2.3.2.1
 - Strain name: unknown
 - Maybe used in 2012- in the north



Difference in antigenicity among H5N1 viruses of different clades and sub-clades

HI antigen		HI antibody titer (log2) of pooled anti-sera to		
HA clade	H5 virus	Re-1Re-5(clade 0)(clade 2.3.4)		Re-6 (clade 2.3.2)
1.1	A881/2010	7	6	3
	A1192/2012	8	7	3
2-3-2-1-A	A675/2011	7	5	7
	A1207/2012	7	5	7
2-3-2-1-В	A672/2011	3	4	8
	A1163/2012	2	3	7
2-3-2-1-C	A1544/2012	5	5	8
	A1648/2012	5	5	8

Summary outcome of vaccine efficacy test in chicken

Challenge virus (HA clade)	Mortality after challenge of chickens vaccinated twice with;			
	Re-1 (clade 0)	Re-5 (clade 2.3.4)	Re-6 (clade 2.3.2.1)	
1.1	0%	0-10%	50%	
2.3.2.1.A	0%	0%	0%	
2.3.2.1.B	50%	70-90%	0%	
2.3.2.1.C	ND	ND	0%	

ND; not done

NEWS

Indonesia says it has found more virulent bird flu strain



Indonesia has identified the bird flu virus that killed hundreds of thousands of ducks in recent weeks as a more virulent type which is new to the country, according to a letter seen on Tuesday. -- PHOTO: RAHIM ESA

JAKARTA— (AFP) Indonesia has identified the bird flu virus that killed hundreds of thousands of ducks in recent weeks as a more virulent type that is new to the country, according to a letter seen Tuesday.

"We found a highly pathogenic avian influenza sub-type H5N1 (virus) with clade 2.3...," the agriculture ministry's veterinary chief Syukur Iwantoro said in the letter obtained by AFP.

"This clade is a new clade found for the first time in Indonesia, that is very different to the avian influenza found before, which is clade 2.1."

Thoughts on Why clade 2321 virus are expanding?

HA clade 2-3-2-1 virus crossed the borders of South, Southeast, and East Asia in 2009-11 Clade 2-3-4 could not, but 2-3-2-1 can, Why?



Clade 2-3-4 was the major clade in China during 2006-2009, but did not spread to South nor East Asia

Clade 2-3-2 has expanded to South & East Asia and Europe during 2009-20011. Who is carrying the virus?

LETTER

doi:10.1038/r

Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

Masaki Imai¹, Tokiko Watanabe^{1,2}, Masato Hatta¹, Subash C. Das¹, Makoto Ozawa^{1,3}, Kyoko Shinya⁴, Gongxun Zhong¹, Anthony Hanson¹, Hiroaki Katsura⁵, Shinji Watanabe^{1,2}, Chengjun Li¹, Eiryo Kawakami², Shinya Yamada⁵, Maki Yasuo Suzuki⁶, Eileen A. Maher¹, Gabriele Neumann¹ & Yoshihiro Kawaoka^{1,2,3,5}

Question: Would the molecular changes in HA gene allow a virus possessing subtype H5 HA to be transmissible among mammals?

Result: We identified a reassortant H5 HA/H1N1 virus (comprising H5 HA with four mutations and the remaining seven gene segments from a 2009 pandemic H1N1 virus) that was capable of droplet transmission in a ferret model. The transmissible H5 reassortant virus preferentially recognized human-type receptors, replicated efficiently in ferrets, caused lung lesions and weight loss, but was not highly pathogenic and did not cause mortality.

Discussion: These results indicate that H5 HA can convert to an HA that supports efficient viral transmission in mammals; however, we do not know whether the four mutations in the H5 HA identified here would render a wholly avian H5N1 virus transmissible.

Four mutations on H5 gene enabled reassortant H5 HA/H1N1 virus to be capable of droplet transmission in ferrets.



(from Kawaoka's paper)

How many of HPAI H5N1 viruses 1996-2011 has those 4 mutations in HA gene?

 2978 HA sequences from public database that covers viruses from 1996 till early 2011 were examined for the presence of 4 mutations that were mentioned in Kawaoka's paper.

Mutation 1: N158D

•780 of 2978 (26%) had this mutation.

- Mutation 2: N224K
 - •1 out of 2978
- Mutation 3: Q226L
 - •None of 2978
- Mutation 4: T318I

•1 out of 2978

% of HA sequences with specific mutation



Mutation **N158D** was found in **26%** of HPAI H5N1 viruses Which HA clades of H5 viruses have "mutation **N158D**"?

Mutation N158D was found in

- Clade 0: 14/120 (12%)
- Clade 2.2.X 679/1015 (67%)
 - 2.2 337/539 (63%)
 - 2.2.1 301/380 (79%)
 - 2.2.2.1 41/96 (43%)
- Clade 2.3.2.1 73/86 (85%)
- The other clades: almost none

Mutaion N158D was found mainly in clade 2.2.X and 2.3.2.1.



Number of HA sequences

N158D mutation was found mainly in clade 2.2 and 2.3.2.1 viruses

What are the common feature of clade 2.2 and 2.3.2.1 in comparison with the other clade?



Clade 2.2

Clade 2.3.2.1

Both clade 2.2 and 2.3.2.1 has expanded widely across the regions while the other clades did not.

What are the effect of "mutation **N158D**" on H5 viruses?



Figure 6 | Effect of heat treatment on the infectivity and haemagglutination activity of viruses. Aliquots of a virus stock containing 128 HA units were incubated for the times indicated at 50 °C. a, Virus titres in heat-treated samples were determined by plaque assays on MDCK cells. b, Haemagglutination titres in heat-treated samples were determined by using haemagglutination assays with 0.5% TRBCs. Each point represents the mean ± standard deviation from triplicate experiments.

 The viruses with N158D mutation are shown to have **higher heat stability** than the ones without this mutation.

(from Kawaoka's paper)

One example HA clade 2-3-2-1 virus crossed the borders of South, Southeast, and East Asia in 2009-11 Clade 2-3-4 could not, but 2-3-2-1 can, Why?



Clade 2-3-4 was the major clade in China during 2006-2009, but did not spread to South nor East Asia

N158D mutation: NO (1/463)

Clade 2-3-2 has expanded to South & East Asia and Europe during 2009-20011. Who is carrying the virus?

N158D mutation: Yes (73/86)

One more example Even within Clade 2.3.2 viruses



Summary

- Nine epi-zones were identified based on virus character, geographical location, and the movement of birds.
- HPAI surveillance and control should be based on epi-zone by epi-zone instead of country by country.
- More coordination and cooperation needed among countries

Thank you

Essential Elements for Surveillance of Avian Influenza at the Domestic Poultry/Wild Bird Interface



David E. Swayne

Southeast Poultry Research Laboratory

Agricultural Research Service U.S. Department of Agriculture Athens, Georgia





Swayne, D.E. Epidemiology of Avian Influenza in Agricultural and Other Man-Made Systems. In: Avian Influenza. Wylie-Blackwell (www.blackwellpublishing.com), 2008.

Surveillance

- Where is the virus?
 Using scientific principles to design sampling and select test methods to answer the question
 - a. Ecology/epidemiology
 - **b.** Host species
 - c. Agricultural production systems
 - d. Geographic distribution
- 3. Resulting information is used to develop control/eradication strategy
- 4. Continual assessment

LPAIV Ecology and Epidemiology



• The vast majority of LPAI viruses in free-living birds never enter poultry and agricultural systems

AIV Epidemiology and Pathobiology Concepts



• Adaptation: progressive viral genetic changes that result in increasing efficiencies of replication in a host species

• Infection: low to high infectivity

• **Pathogenicity** ability to produce lesions, disease and/or death in a host directly - associated with quantity of virus replication

Transmissibility – natural host-to-host spread

32 HPAI Disease Events

1. 1959: Scotland, H5N1 2. 1961: S. Africa, H5N3 3. 1963: England, H7N3 4. 1966: Canada, H5N9 5.1975: Australia, H7N7 6. 1979: Germany, H7N7 7. 1979: England, H7N7 8. 1983-84: USA, H5N2 9. 1983: Ireland, H5N8 10. 1985: Australia, H7N7 11. 1991: England, H5N1 12. 1992: Australia, H7N3 13. 1994: Australia, H7N3 § 14. 1994-95: Mexico, H5N2 § 15. 1995 & 2004: Pakistan, H7N3 16. 1997: Australia, H7N4 17. 1997: Italy, H5N2 * § 18. 1996-2012: Eurasia/Africa, **H5N1**

19. 1999-2000: Italy, H7N1 20. 2002: Chile, H7N3 21. 2003: Netherlands, H7N7 22. 2004: USA, H5N2 23. 2004: Canada, H7N3 24. 2004, 2006: S. Africa, H5N2 (ostriches) § 25. 2005: N. Korea, H7N7 26. 2007: Canada, H7N3 27. 2008: England, H7N7 28. 2009: Spain, H7N7 29. 2011: S. Africa, H5N2 (Ostriches) 30. 2012: Chinese Taipei, H5N2 § 31. 2012: Mexico, H7N3 32. 2012: Australia, H7N7 *Largest epizootic in 50 yrs § Vaccine used in the control strategy

H5N1 HPAIV Ecology and Epidemiology



- H5N1 HPAI has different ecology in wild birds than LPAIV
- Different risks with different agricultural production systems

Agricultural Production in Asia

Sectors 1 and 2 – Industrial Sector

- Concentrated production high populations; few farms but each with large poultry populations
- Biosecurity is usually good and access is controlled
- Best control of disease and most consistent vaccine coverage





Poultry Production Features

- Sector 3 independent 'commercial' but not integrated industrial
 - **Biosecurity is limited**
 - Movement controls and veterinary care variable to minimal
 - Major producer system in mainland Asia
 - **Re-infections are common place**
 - Vaccine coverage inconsistent to poor







Poultry Production Features

Sector 4 – Village Poultry

- Up to 50% of Asian country's production
- Lack of movement controls
- "Syndromic surveillance"
 - Adequate vaccination coverage not achieved
- **Rapid movement of birds to and from LPM**



Poultry Production Features

- Sectors 3 & 4 Large populations of Domestic Ducks and Geese
 - 1° Outdoor reared
 - Asymptomatic infection
 - Major reservoir and biomass issue







Role of Wild birds in Asia

- Recovery H5N1 from dead wild aquatic birds (sporadic and epizootic), few asymptomatic infections detected in surveys
- Periurban birds (e.g. tree and house sparrows, starlings, pigeons...) – can be mechanical and at times biovectors
- The issues for Asia are:
 - Numbers: 20-30 million migratory Anseriformes verses 20 billion poultry
 - Sector 3 & 4: a billion households with poultry; no vaccination, no movement controls, minimal surveillance
 - Find the reservoir in endemic countries?
 - Introduction verses secondary spread







Stallknecht, FAO/OIE, 2006 Wild birds can be infected with H5N1 HPAIV But, a virus isolation does not make a reservoir (nor the Major Risk)!



H5N1 HPAI control and eradication will require enhanced surveillance to identify the reservoirs and sites of action Role wild bird surveillance:

- 1. Passive dead bird to find incursion in area leading to increased surveillance
- Refocus on domestic ducks (significant reservoir) in area as environmental sentinels
 LPM surveillance

What are the high risk activities?



Sources for Virus

Sector 1-3 Infected Commercial Poultry Wild waterfowl



Domestic Ducks

Sectors 1-3 Naïve Commercial Poultry Clothing, shoes & equipment (Mechanical)



Village Chickens

LPM

Interface: Wild Birds/Agriculture

- Sector 3 & 4 must be part of surveillance
 Targeted surveillance:
 - a. LPM and epidemiologic linkages of suppliers
 - **b.** Domestic duck and geese populations
 - c. Vaccinated poultry populations
 - d. Enhancements when HPAI detected
- 3. Need some movement controls: one-way movement
- 4. Market chain analysis

Thank You For You Attention!

