AI Vaccine And Immunization

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Ⅰ. The Pathogen

Influenza Virus

Type A （Avian, Human, Mammals）

Causing endemics or Pandemics

Type B （Human）, Local Endewics

Type C （Human, Pig）, Sporadically endemics

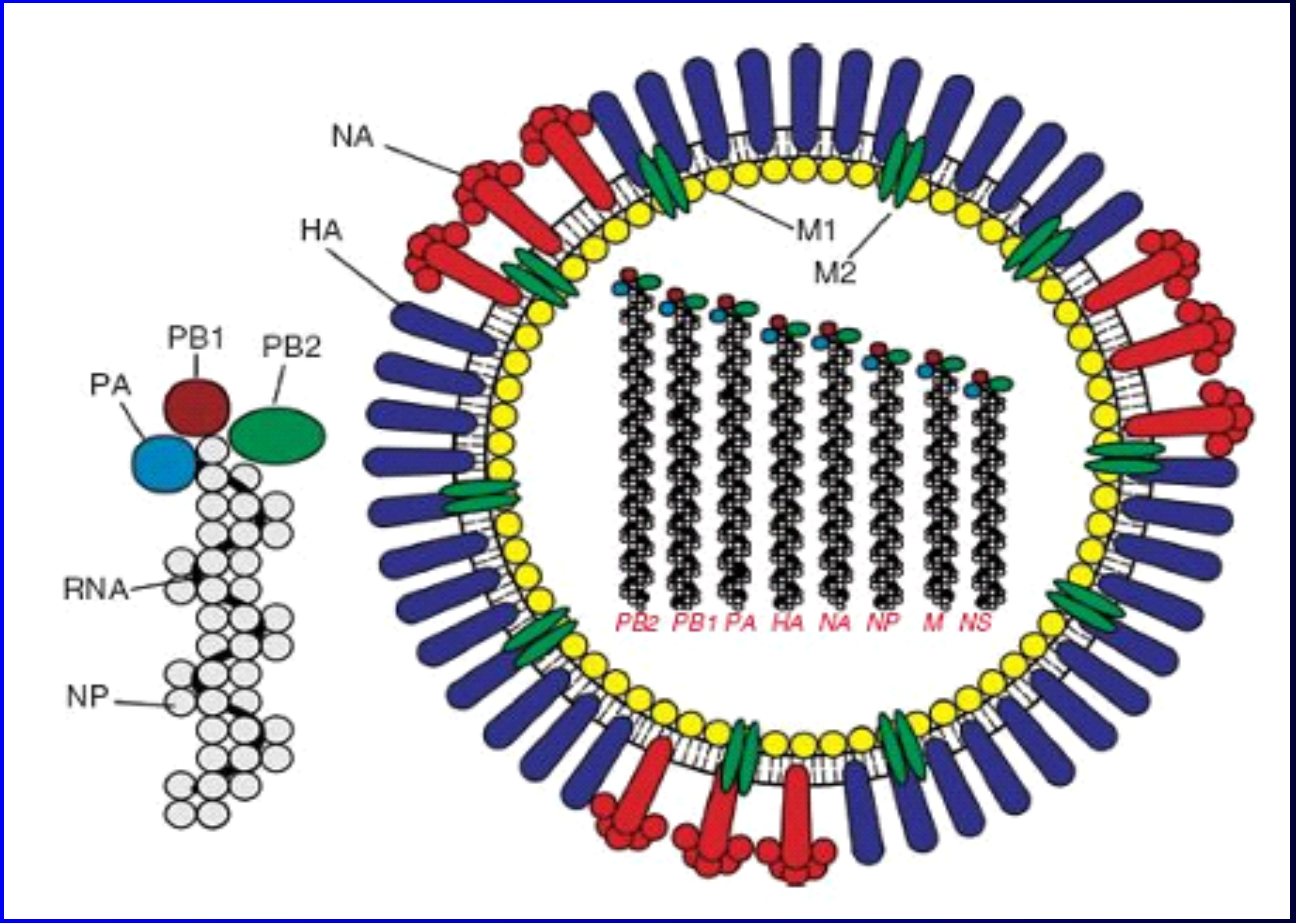
Schematic representation of influenza A virus

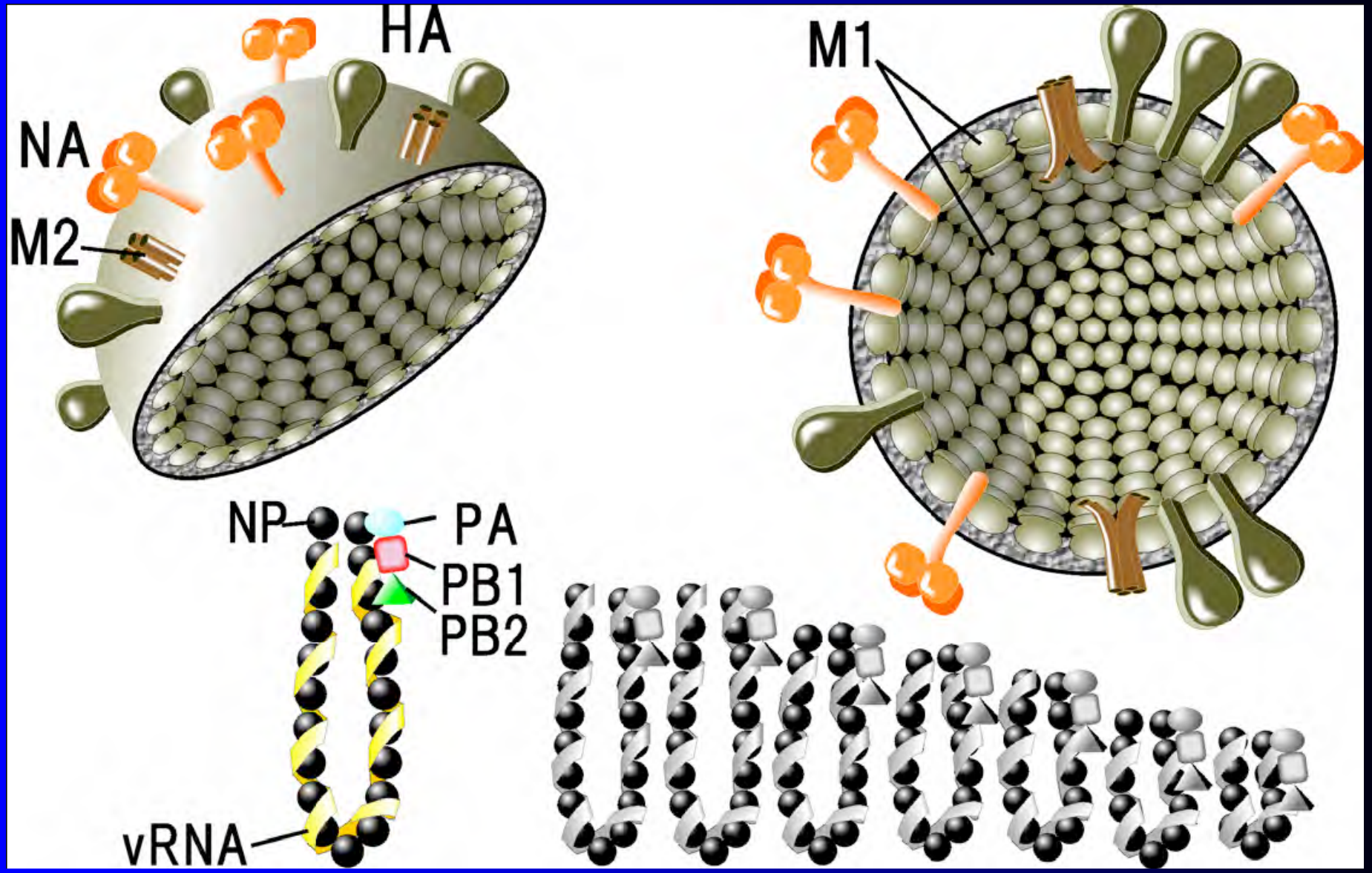
HA:

H1-H16

NA:

N1-N9

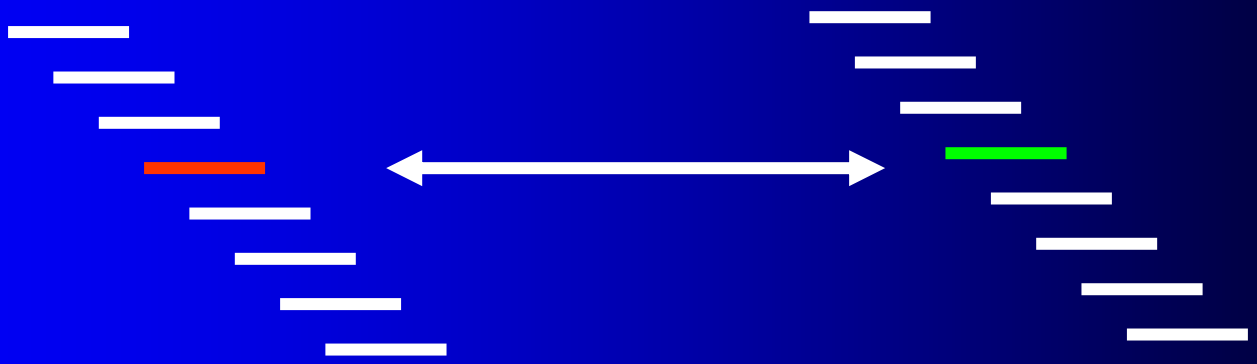




HA：NA=75：25

The AIV RNA has 8 independent fragments. This structure is in favor of gene assortment between different strains and virulence change.

H5N1 （Avian） H1N（Human）



Based on its pathogenicity, the AIV can be divided into highly pathogenic avian influenza virus (HPAI, H5N1), low pathogenic avian influenza virus (LPAI, H9N2) and non-pathogenic avian influenza virus.

B-X-B-R（B-Basic arginine or lysine, X-non-basic aminoacid，R-Arginine）is the minimal sequence of the highly pathogenic virus.

	H5 subtype	H7 subtype	H9 subtype
Highly pathogenic	RERRRKKR/G	PKRKRKR/GL	——
Low pathogenic	PQRETR/GL	PENPKGR/GL	PARSSR/GL

The virulence of AIV is determined by whether the amino acid of the HA protein is liable to be cleaved in vivo. Particular attention should been given to

HA----------HA1+HA2

Tryptase

E.coli

Staphylococcus

Ⅱ. Avian Influenza Vaccines

(1) Inactivated Oil Emulsion Vaccines

(2) Gene Modified Live Vaccine

(1) Inactivated Oil Emulsion Vaccines

Avian Influenza Vaccine (H5N2, N28 strain) —for exportation only

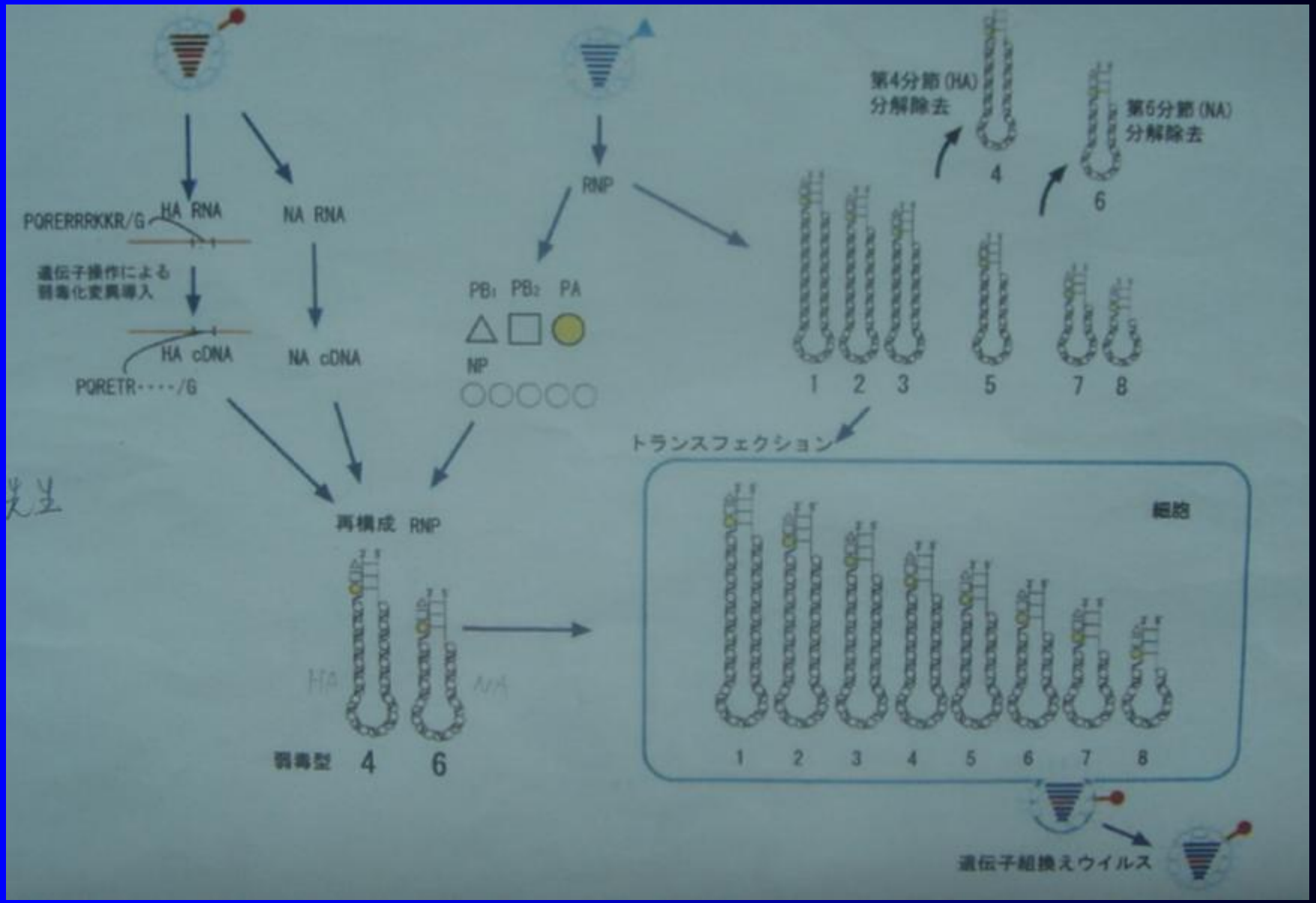
Reassortant Avian Influenza Vaccine, Inactivated (H5N1, RE-1 strain) —Southern Provinces

Reassortant Avian Influenza Vaccine, Inactivated (H5N1, RE-1 strain+ RE-4 strain) —13 Northern provinces

Bivalent Avian Influenza Vaccine, Inactivated (H5+H9, RE-1+RE-2 strain) —Throughout the country

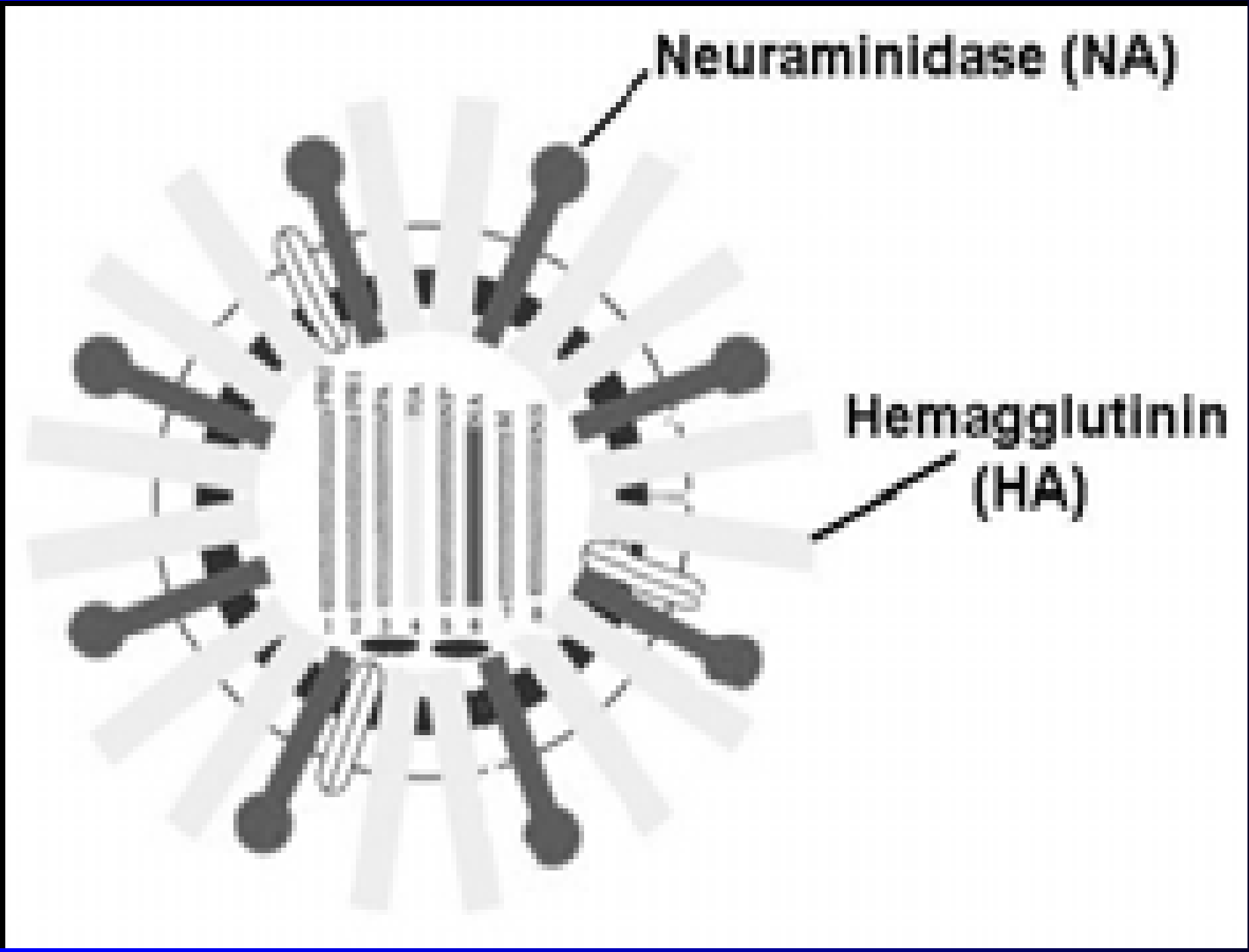
Avian Influenza Vaccine, Inactivated (H9N2, F stain) —Throughout the country

Avian Influenza and Newcastle Disease Vaccine, Inactivated (La Sota+ F strain) —Throughout the country



RE Vaccine: Fragment 4 encodes the HA;

Fragment 6 encodes the NA;


Neuraminidase (NA)
Hemagglutinin
(HA)

A Comparison of H5N1 RE-1 and RE-4 Vaccine

	H5N1，RE-1 Vaccine	H5N1，RE-4 Vaccine
Maternal Virus	A/PR/8/34（H1N1）	A/PR/8/34（H1N1）
HA、NA Donor Virus for reassortment	A/Goose/Guangdong/1/1996(H5N1) for short GD/1/96	A/Chicken/ShanXi/2/2006(H5N 1) for short CK/SX/06
Reassortment Methods (See fig. below)	The HA of Fragment 4 and NA of Fragment 6 in A/PR/8/34 virus are replaced respectively with HA and NA genes of GD/1/96 virus	The HA of Fragment 4 and NA of Fragment 6 in A/PR/8/34 virus are replaced respectively with HA and NA genes of CK/SX/06 virus
Antigen Difference	RE-1 antigen test with the HI titer of 2^{7-9} RE-4 antigen test with the HI titer of 2^{1-3}	RE-1 antigen test, with the HI titer of 2^{2-3} RE-4 antigen test, with the HI titer of 2^{7-9}
Use	To prevent avian influenza caused by typical strains, but with poor result to prevent the variant strain infection.	To prevent avian influenza caused by the variant strain.

（2）Gene Modified Live Vaccine

Fowl pox Virus Vectored Assortant Avian Influenza Vaccine, Live

Assortment Avian Influenza and Newcastle Disease Vaccine, Live

Immunizing Methods and Precautions for Inactivated Avian Influenza Vaccine

1. The inactivated oil vaccines can never be frozen, and should be stored at 2~8℃ (within -5 ℃)
2. If there is any demixture , the vaccine can not be used.
3. The vaccine should be pre-warmed in 25 ℃.

Advantages： Low viscosity;

Easy to inject;

Good absorption;

Less irritation.

Immunization Methods

1. Subcutaneous injection in the neck

2. Muscular injection

Subcutaneous injection in the neck：

Subcutaneous injection in the neck (near the back);

Muscular injection in the neck must avoided.

Needles: Gauge 7 needle for the imported MAC adjuvant vaccine; Gauge 8 or 9 needle for the domestic adjuvant vaccine.

Muscular injection：

Superficially muscular injection; Care should be taken to muscular injection in thigh for caged birds.

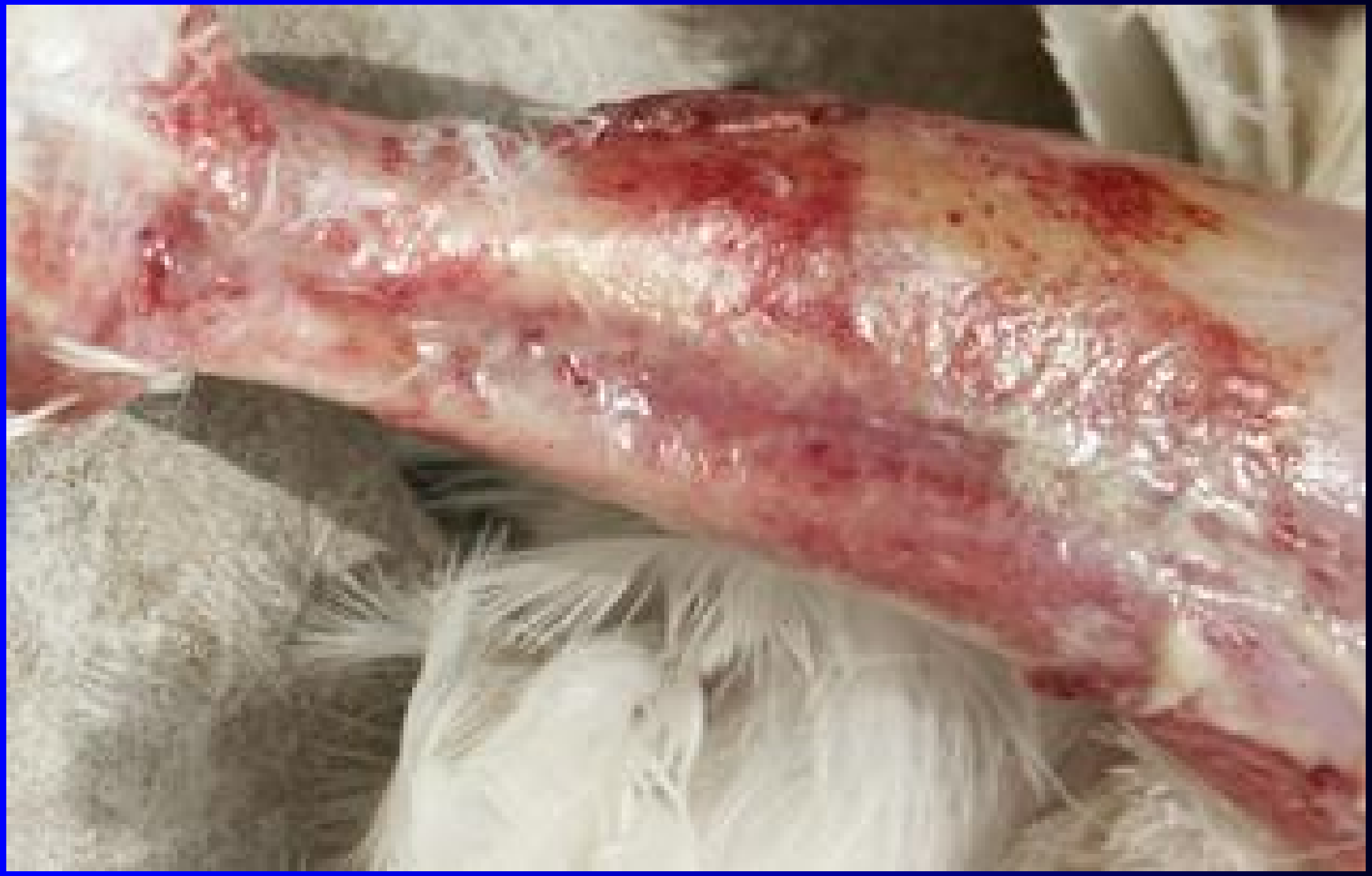
Needles: Gauge 7 needle for the imported MAC adjuvant vaccine; Gauge 8 or 9 needle for the domestic adjuvant vaccine. Needles of over gauge 12 are not allowed.

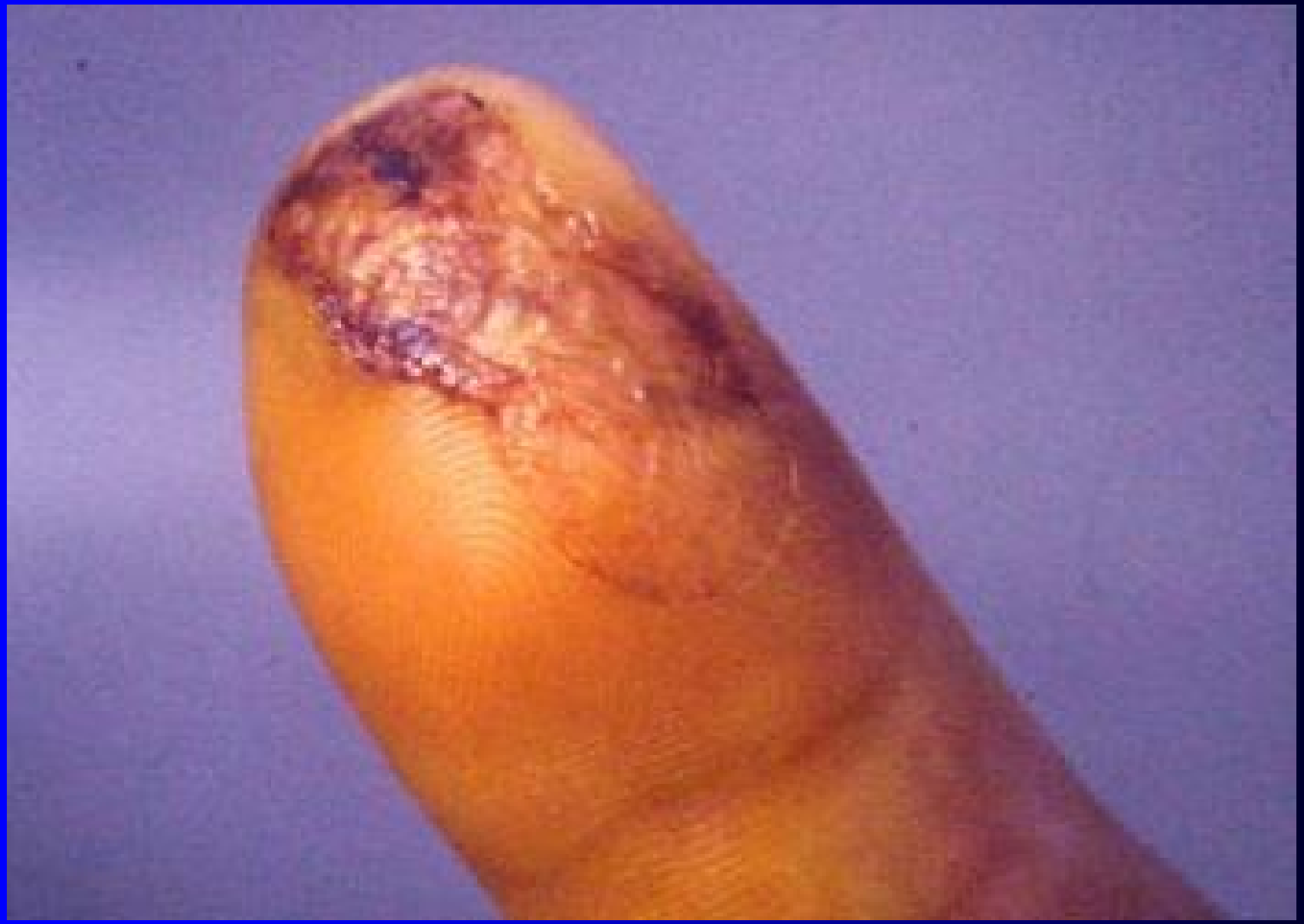

R


I C


R


A I





III. Avian Influenza Immunization

Comprehensive Control Measures

1. To establish bio-security system and healthy flock system with practical operation procedures (SOP).
2. To select quality AI vaccines and work out proper immunization programs according to the practical conditions.
3. To organize the immunization detection unit to monitor the immune status.
4. To understand the disease status and deal with the emergency promptly and resolutely.

1. To establish biosceurity system and healthy flock system is the prerequisite to control avian influenza.

Modern poultry establishments with effective biosecurity system

Backward poultry raising, lack of effective biosecurity system

Free movement of duck - canal/ rivers - floating market

2. To select quality AI Vaccines and work out proper immunization program according to the practical conditions.

The immunization program is determined by AI HI antibody level, when the AI HI antibody is ⩽6 (GMT), immunization should be conducted. If RE-1+RE-4 vaccine or H5+H9 vaccine is applied, test with two antigens should be conducted, and when either one antibody titer (GMT) is ⩽6, immunization should be conducted.

Relations between AI antibody level and protection

	AIV H9			AIV H5			
Vaccine HI antibody	4	5	6	3	4	5	6
Protection(%)	80	90	100	60	80	90	100
Virus shedding	+	+/-	-	+	+	+/-	-
conclusion：The critical AI protection antibody (HI)： (H9)$\geqslant 2^5$; (H5)$\geqslant 2^6$							

3. Immuning Antibody Test

(1) The grass root AI tests mainly include AGID and HI tests.

	AGID	HI
Group specificity	+	—
Type specificity	—	+
Sensitivity	low	high
Comments	no accurate	May be relied

(2) Some mistakes in HI test

1) Red cells of different origins result in significant differences in titer test;

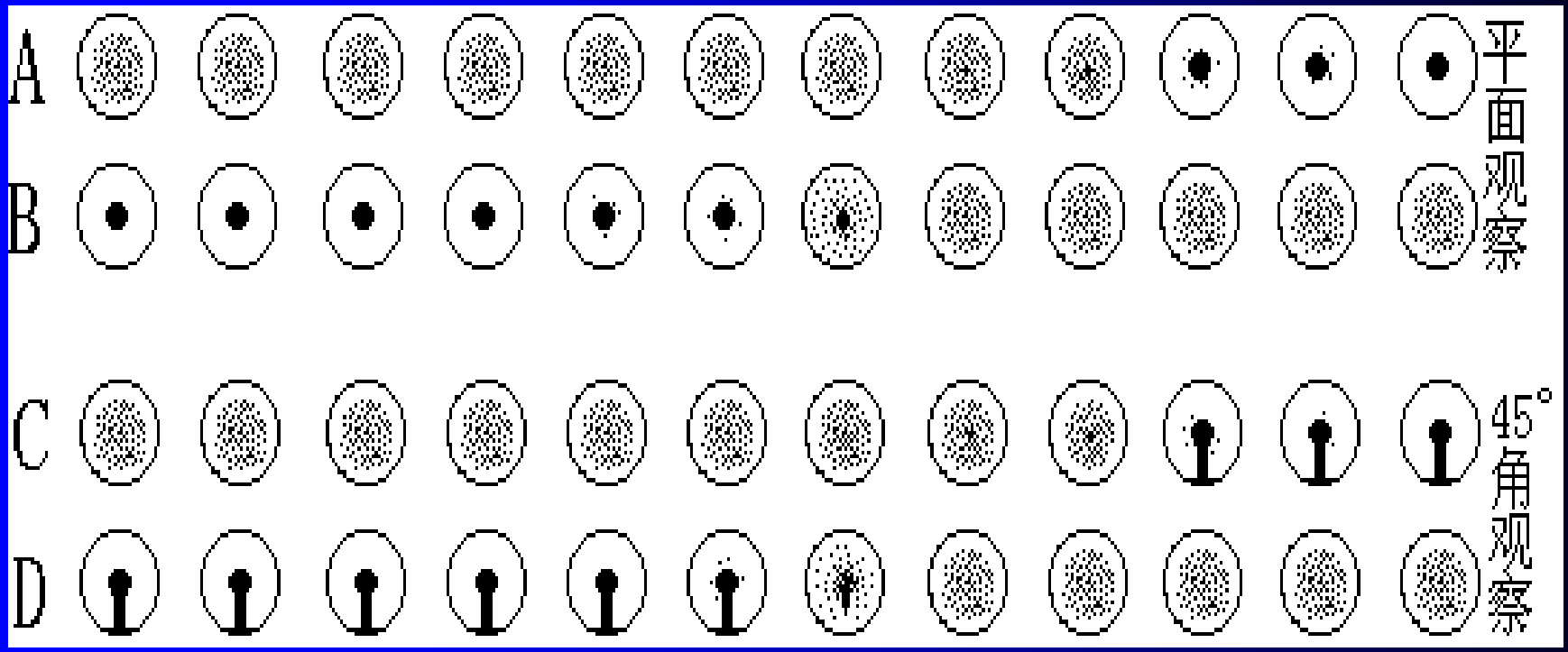
2) Errors in HA titer and working unit standardization result in different HI readings;

3) “Phase” difference in the different AI antigens misleads the practical testing.

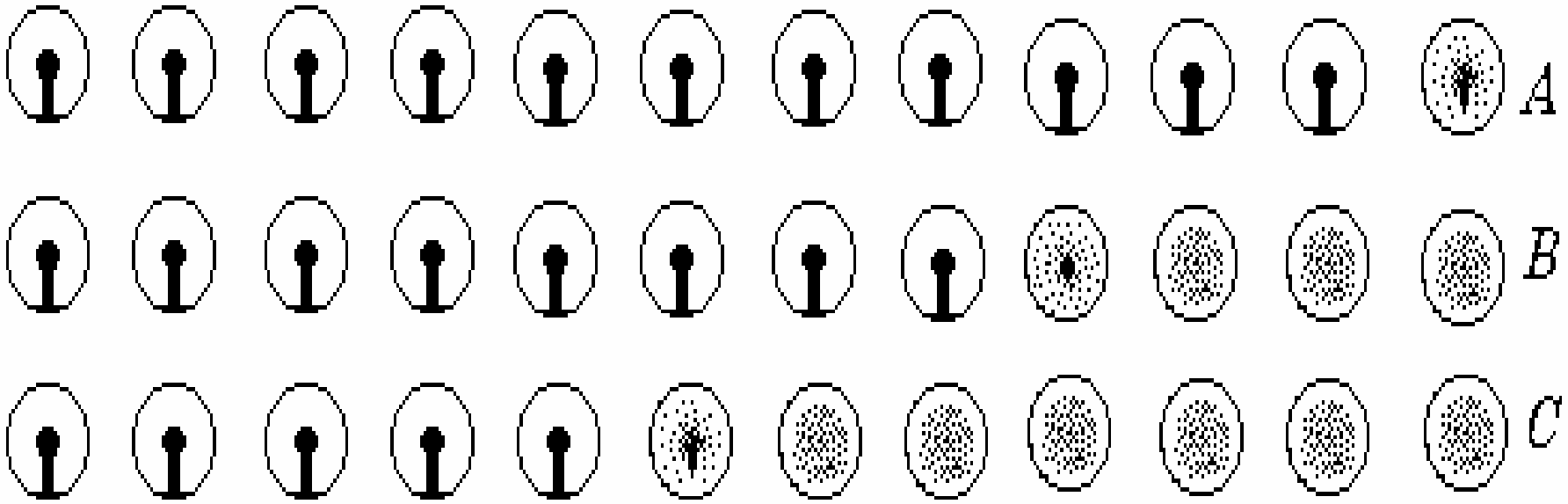
4) Pre-hemagglutination in the water bird sera.

Test results of a serum with different antigens

antigen / serum	"R" Phase	"Q" Phase	"P" Phase
1-P	2^8	2^5	2^9
2-R	2^8	2^6	2^6
3-Q	2^9	2^6	2^5

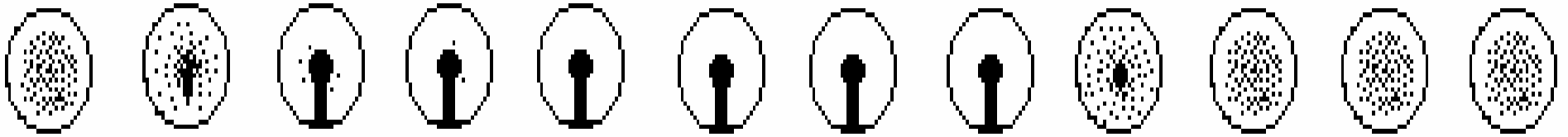


A.C—HA test; B.D—HI test



Errors in HA titer and working unit standardization result in different HI readings.

B-right (2^8), A- too high(2^{11}), C- too low (2^5)



The water bird serum has prehemagglutination, attention should paid to titer reading, the titer is 2^8.

(3) Antibody Test Correction

1) Due to the “Phase ” difference in antigen, each lot of antigen must be tested with at least 3 positive sera and negative control sera, only those with satisfactory results can be used.

2) The test results are valid, when serum titer of negative control well is $<$2 log2, and the difference from the positive control serum well is no more than 1 titer.

3) HI test is negative when HI titer is $\leqslant$3log2; and positive when HI titer is $\geqslant$4log2.

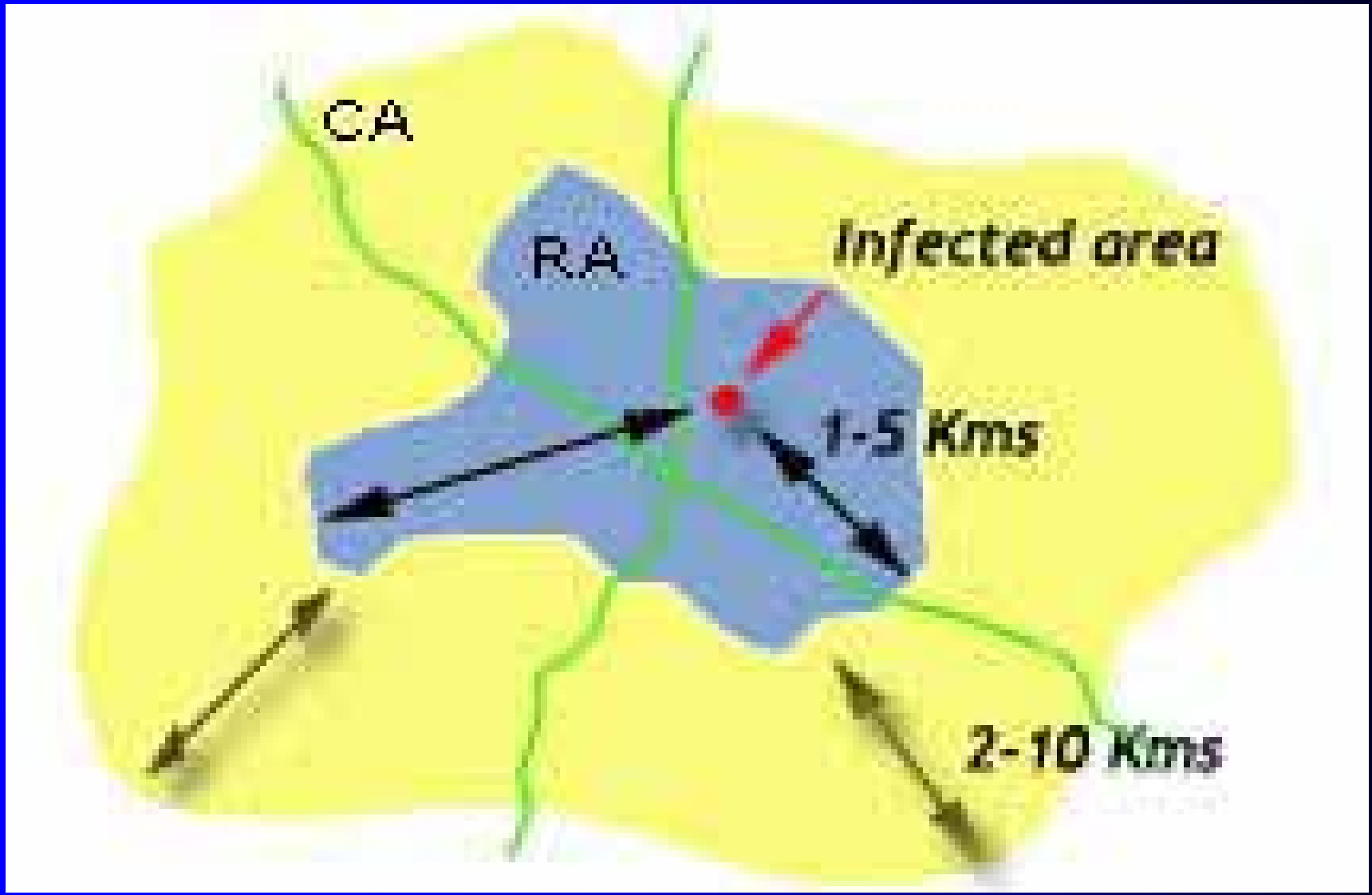
4. To understand the disease status and deal with the emergency promptly resolutely.

Emergent vaccination;

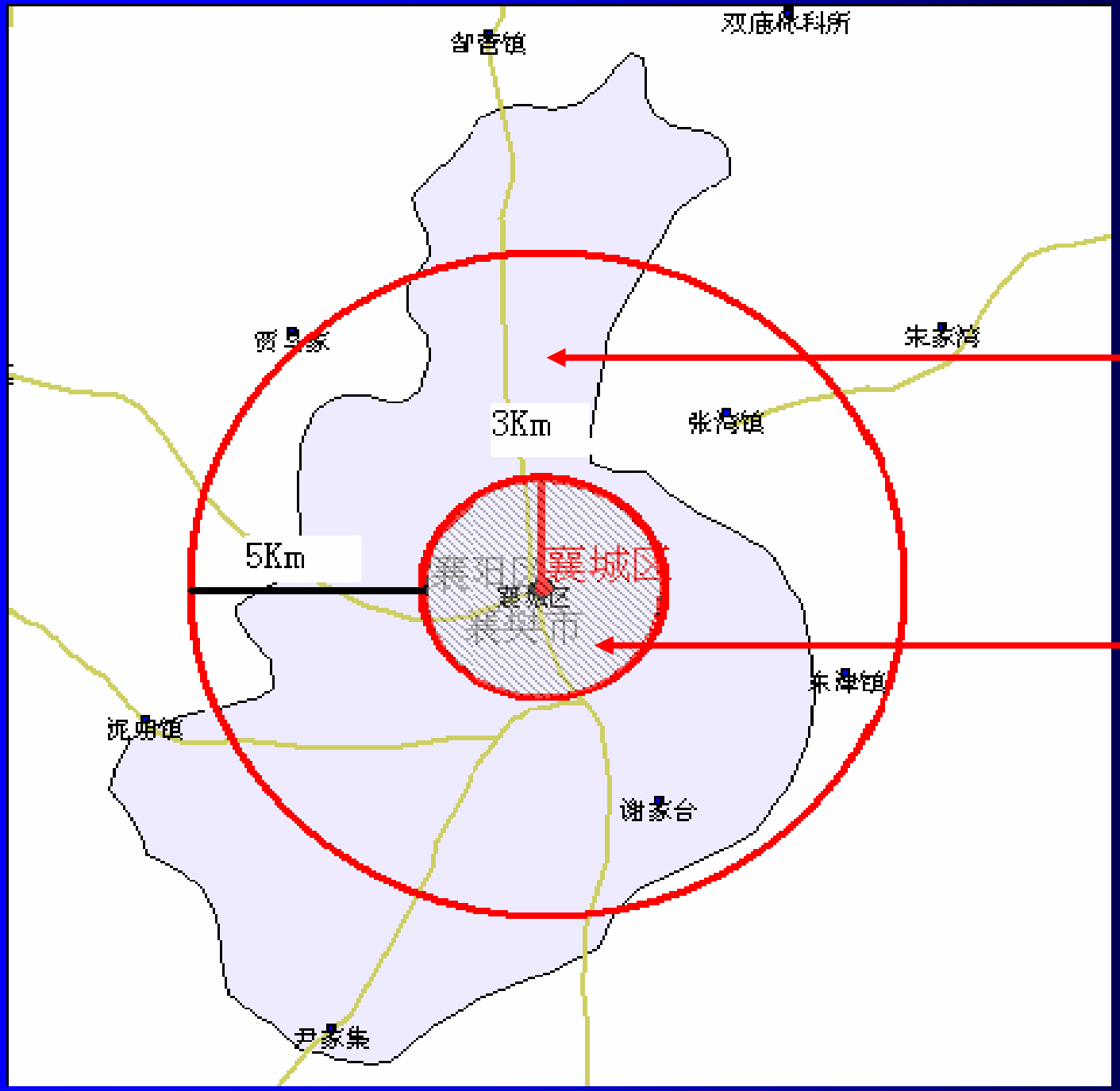
Disinfection, isolation;

Implement emergent programs

……



RA: Restricted area； CA： Control area


双庙林科所
智营镇
贾寨家
朱家湾
张湾镇
3Km
5Km
襄城区
襄阳市
朱滩镇
沈坊镇
谢家台
尹家集
Control area
Restricted area


检查站

Ⅳ. Cause for AI Immunization Failure

1. Chicken Factors

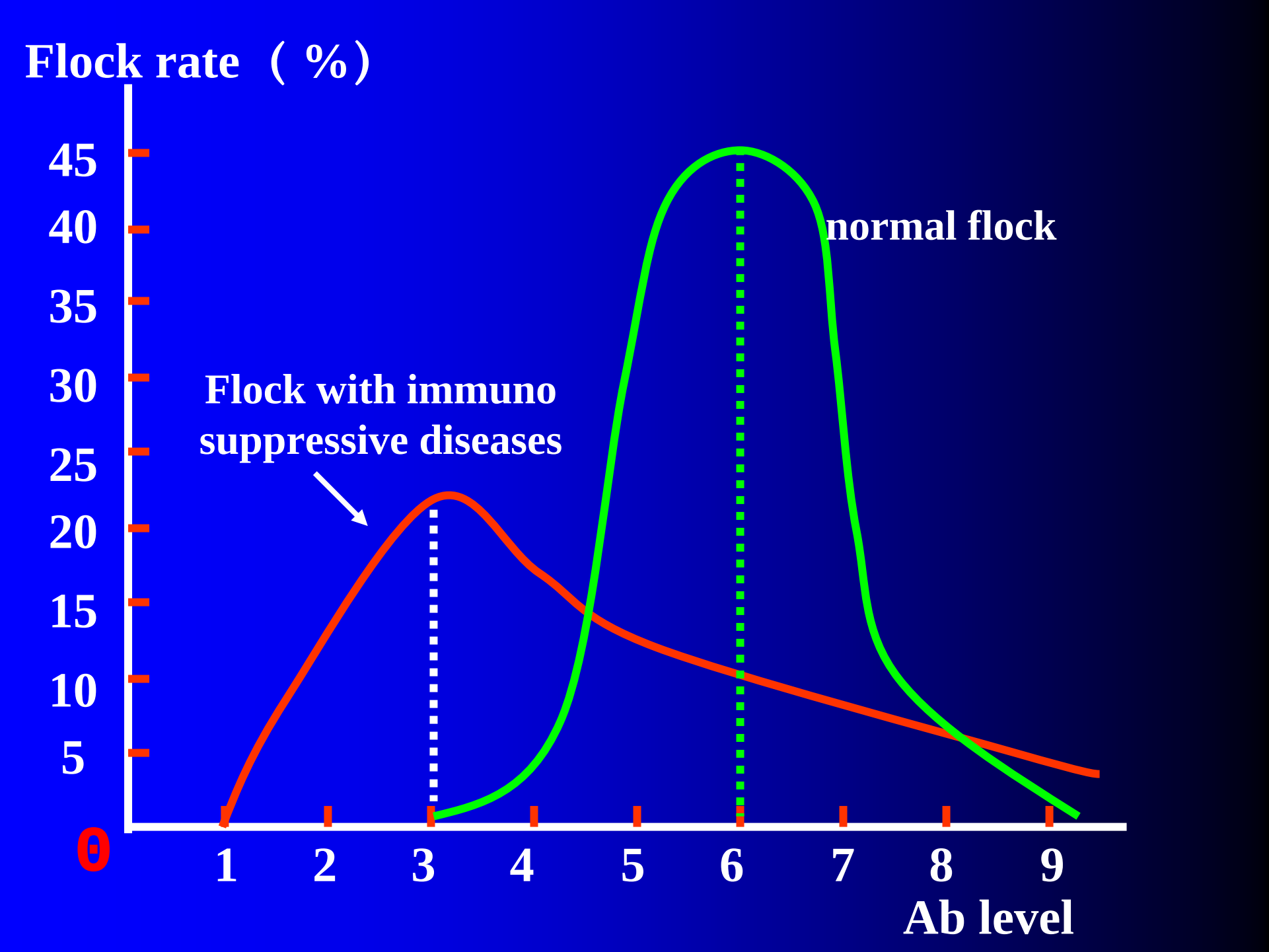
1) Age: when chickens are too young, the immune system has not been completely established, the age must be over 10 days.
2) Maternal antibody: Combine with AI vaccine, and speed up phage digesting process, thus shorten immune duration and delay immunity.
3) Immunosuppression (internal and external diseases)
4) Individual difference: about 2% have no response, and 2% have low response.
5) Chickens with fowl pox vaccine stinging suffer poxes.

Immunization Diseases

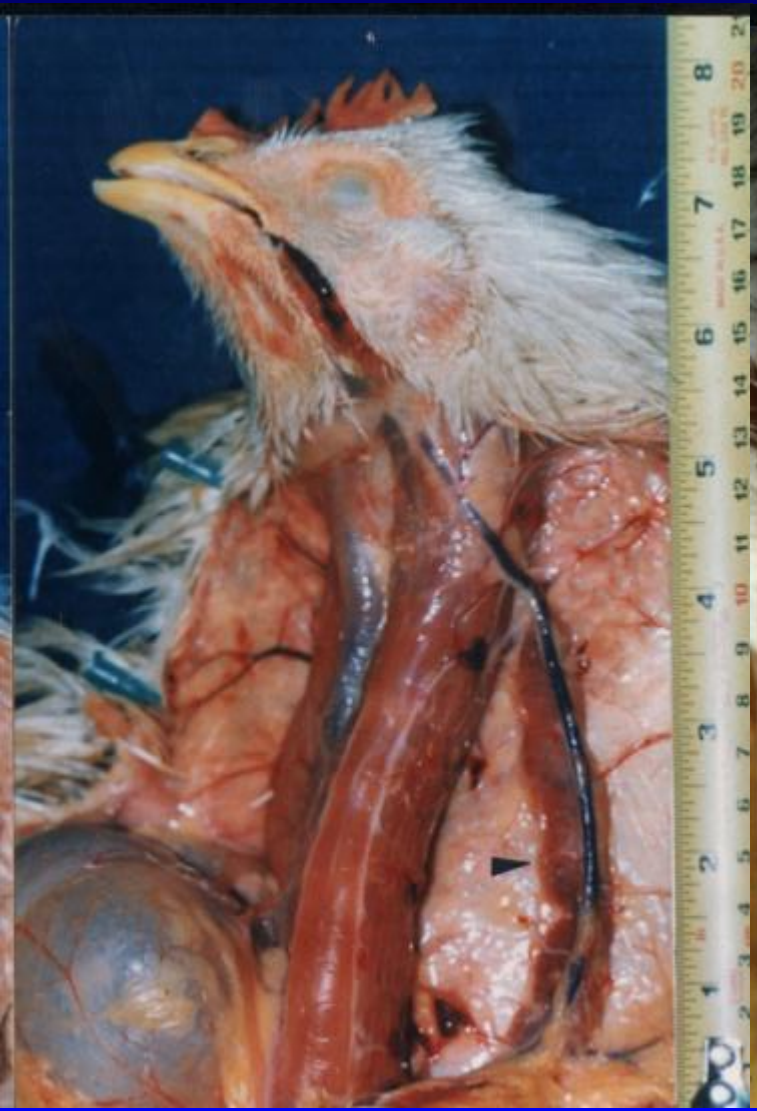
IBD	B cell	humor（Ab)
MD	T	cell+humor
ALV-J	T	cell+humor
CAA	T	cell+humor
REV	B	humor

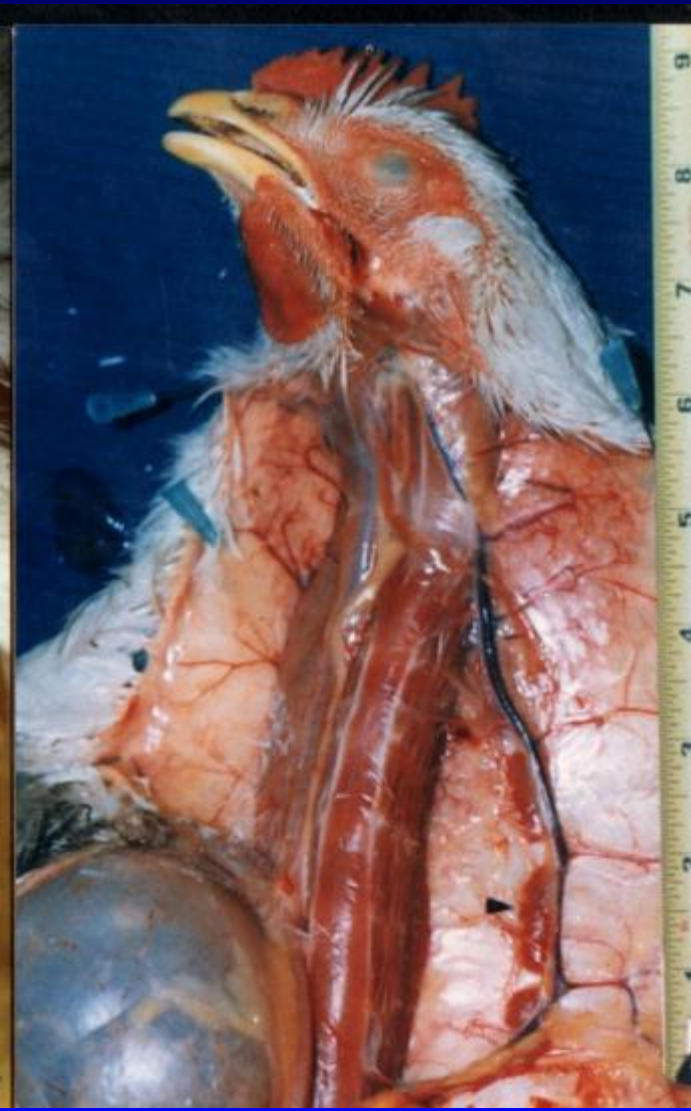
Immuno-suppressive diseases

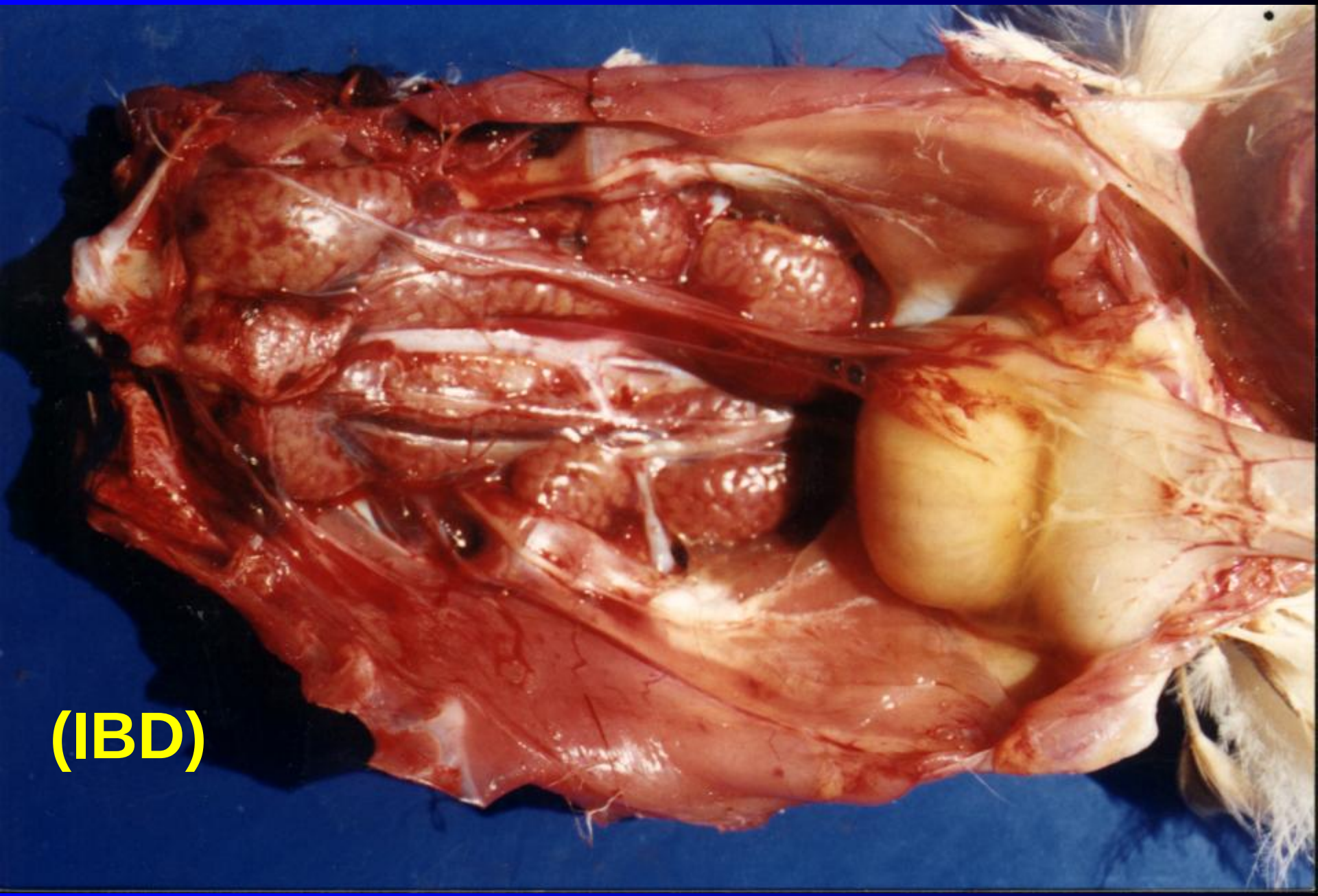
1) Individual “with incomplete immune state”, show low antibody level after immunization, and can not produce effective immune responses’
2) Individual “with incomplete immune state” may become the source of HAPIV infection;
3) Individual “with incomplete immune state” may induce selective antigen variation under immune pressure.


Flock rate（%）
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Ab level
normal flock
Flock with immuno
suppressive diseases

Chicken amenia agent（CAA）






(IBD)


（REV）

2. Serious Environmental Contamination

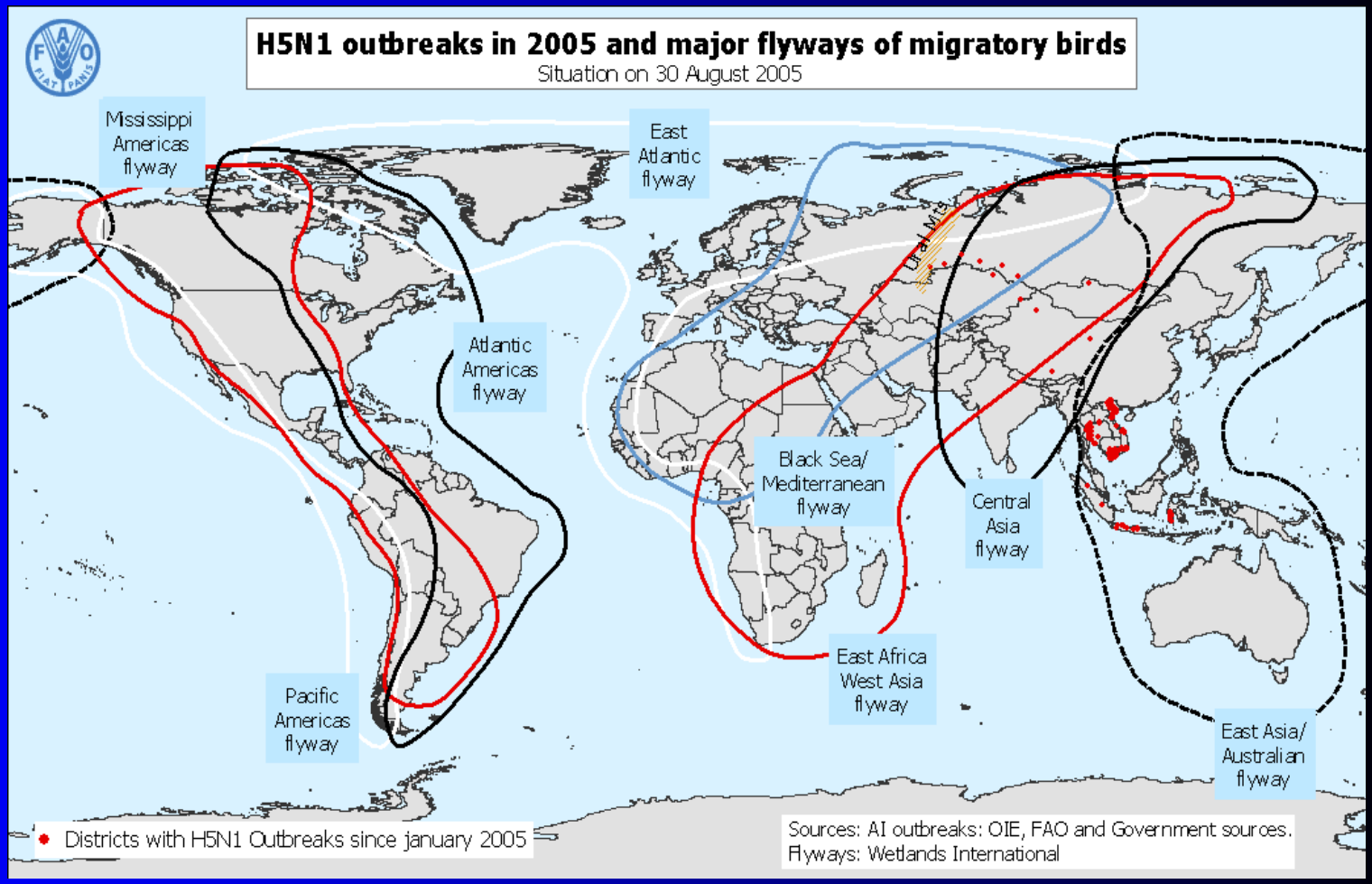
Virus Contamination Effect ! ! !

1) Densely populated, without the effective biosecurity system;
2) Chickens and water birds mixed;
3) Too low immunization coverage.

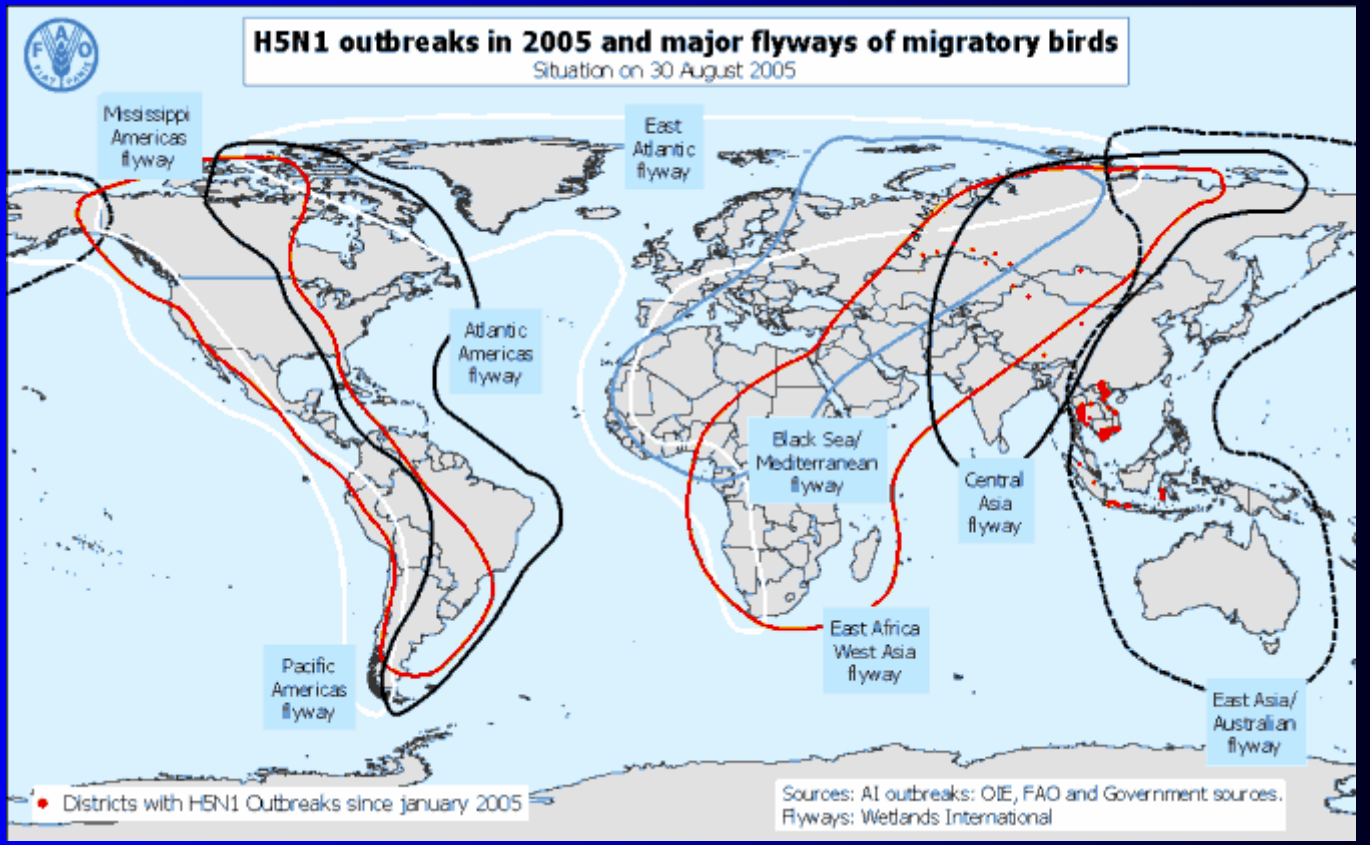
Global distribution of AI (H5N1) outbreaks

Migratory birds spread the virus to every corner of the world

China is covered by the three of eight bird-migration routes



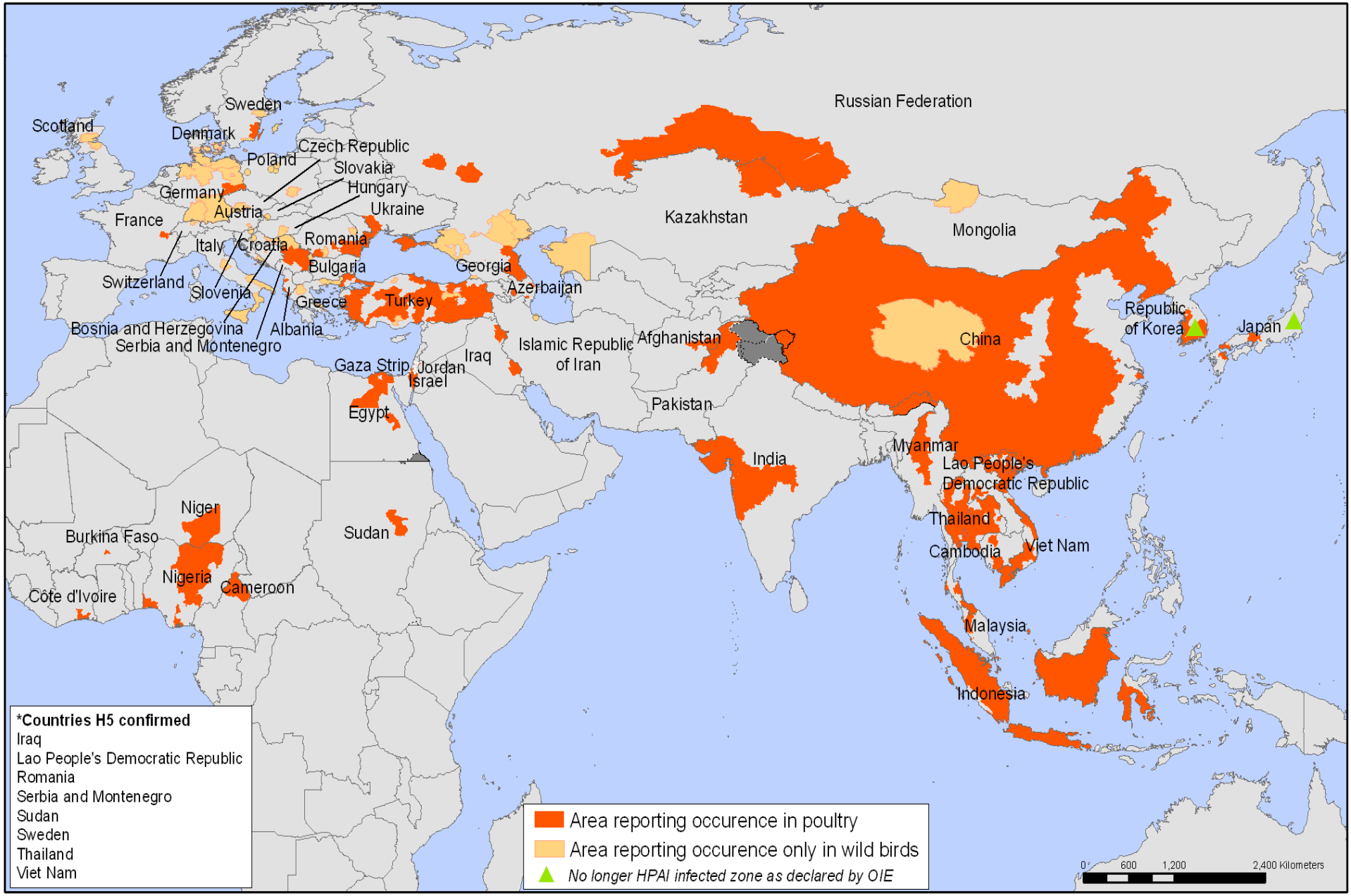
Migratory birds moving routes cover the whole country



The three bird-migration route areas

Areas reporting confirmed occurence of H5N1* avian influenza in poultry and wild birds since 2003

Status as of 28 April 2006



World Health Organization

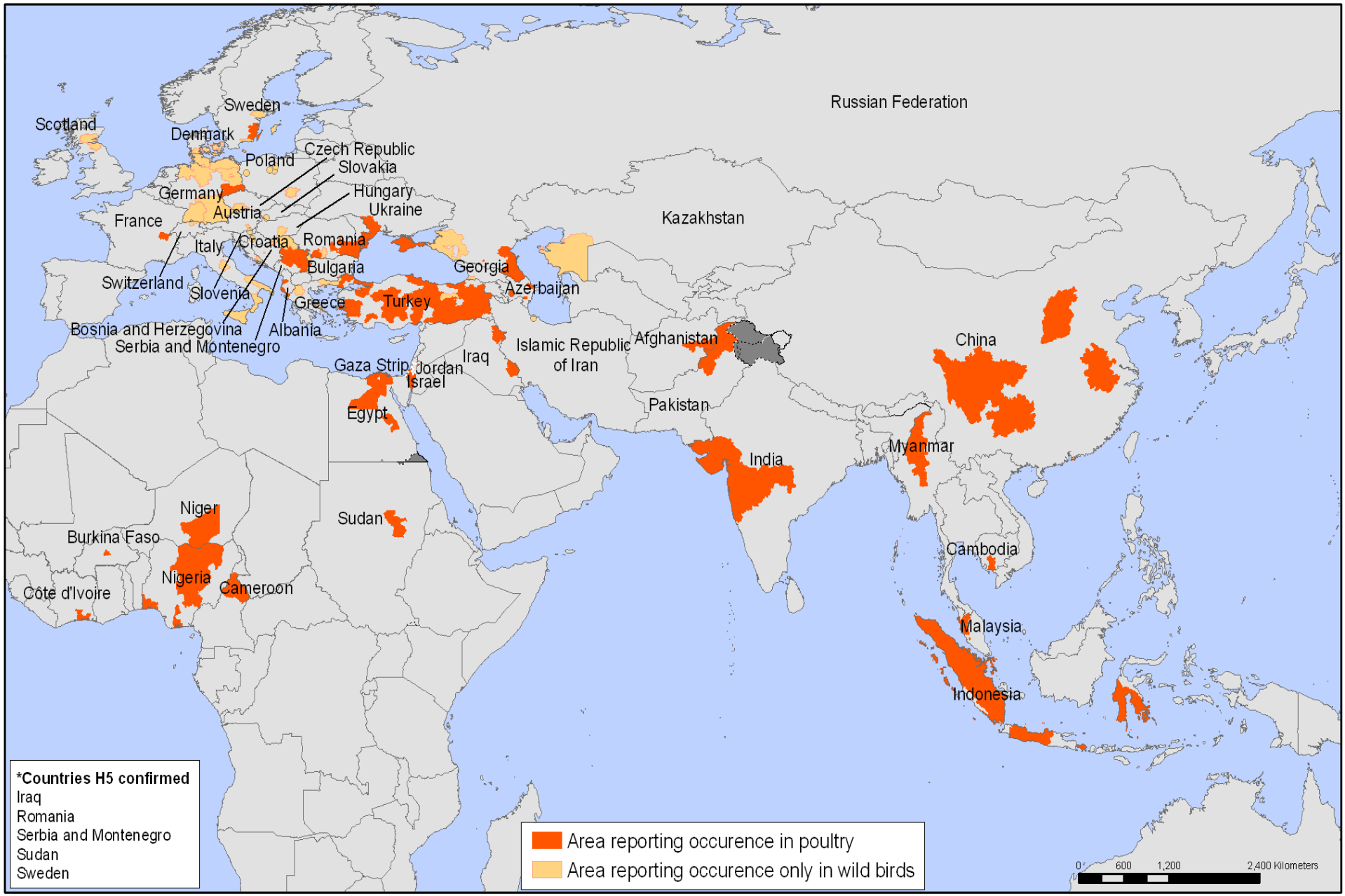
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Organisation for Animal Health (OIE) and national governments

Map Production: Public Health Mapping and GIS
Communicable Diseases (CDS) World Health Organization

Areas reporting confirmed occurence of H5N1* avian influenza in poultry and wild birds since January 2006

Status as of 28 April 2006



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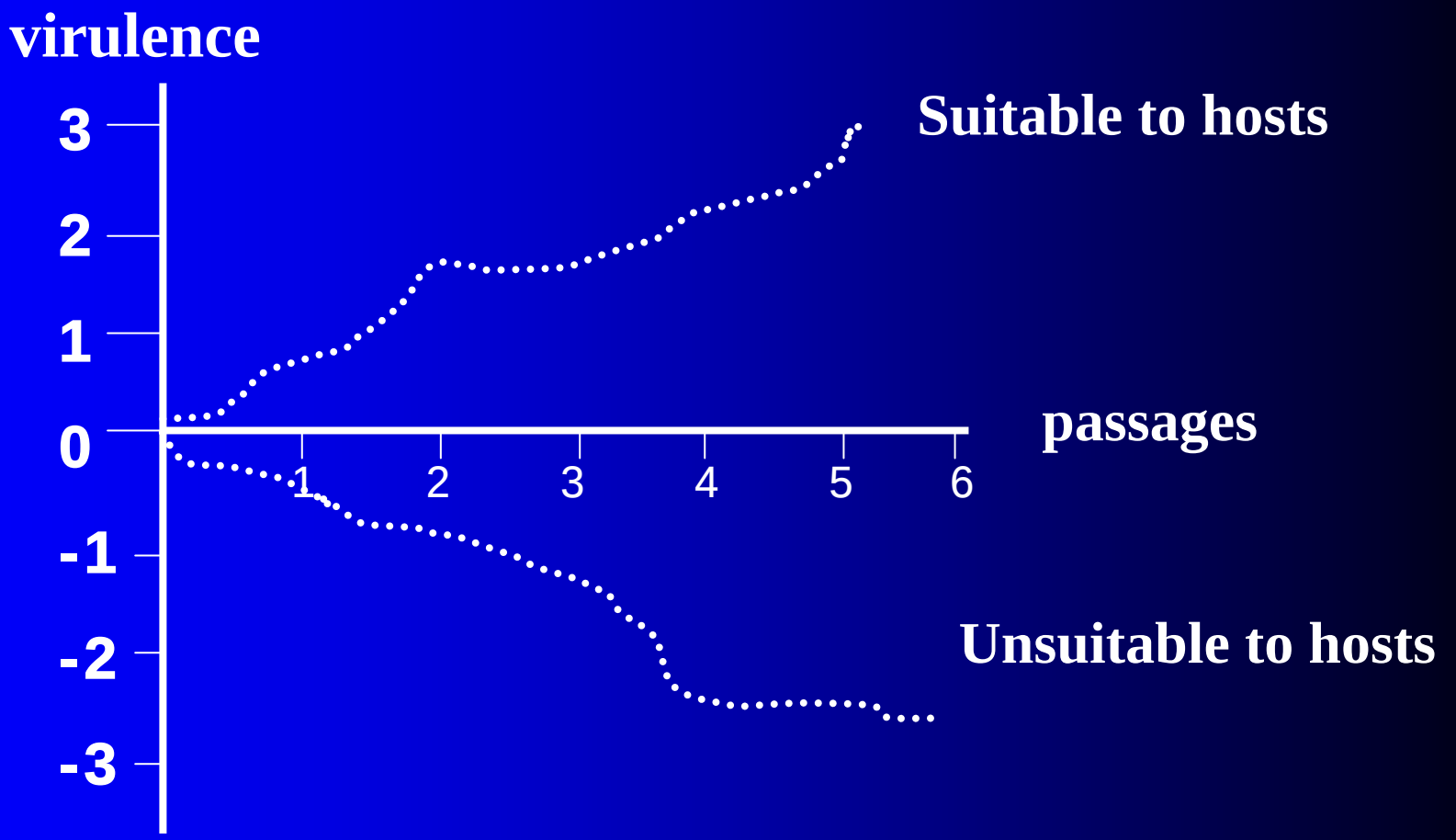
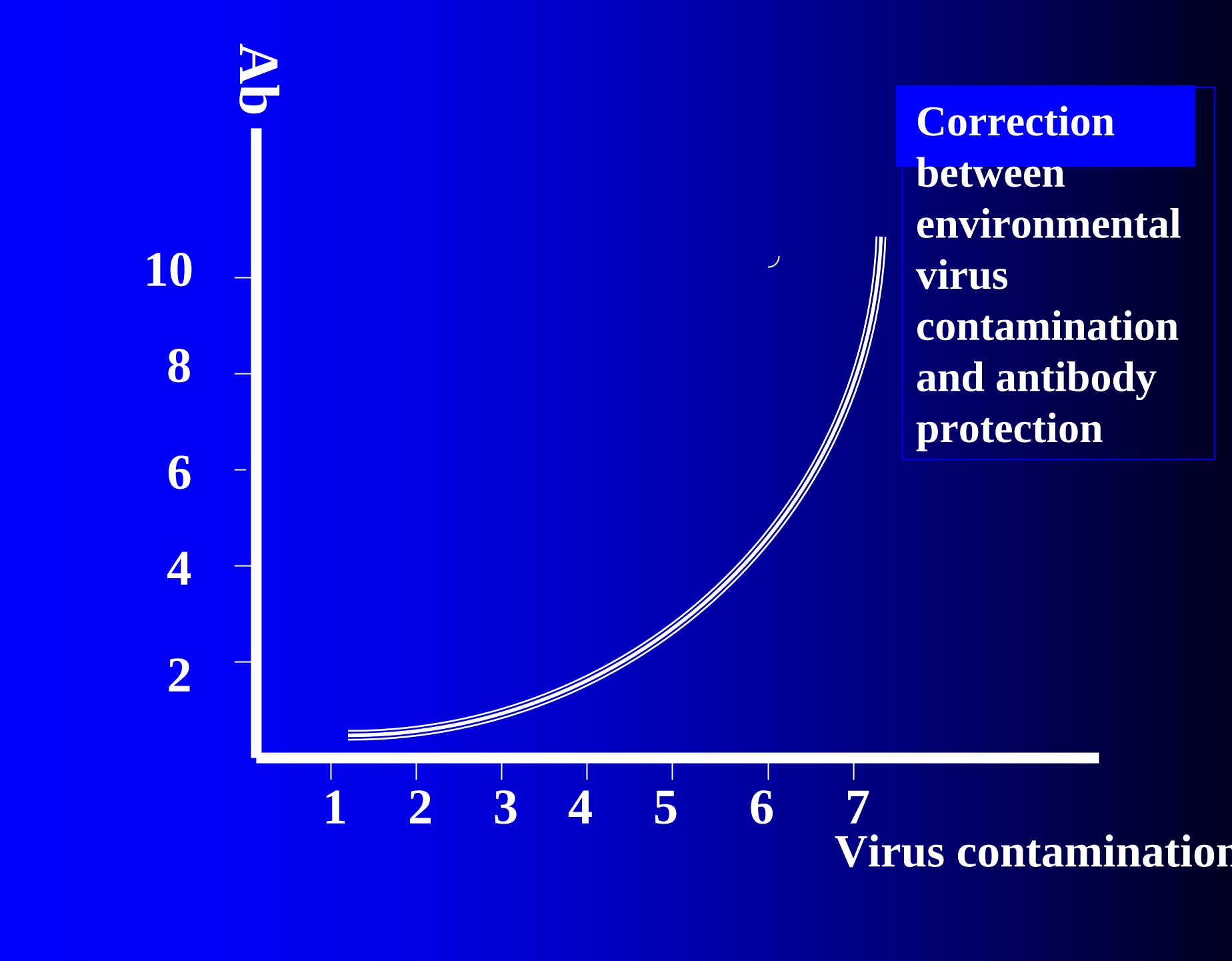
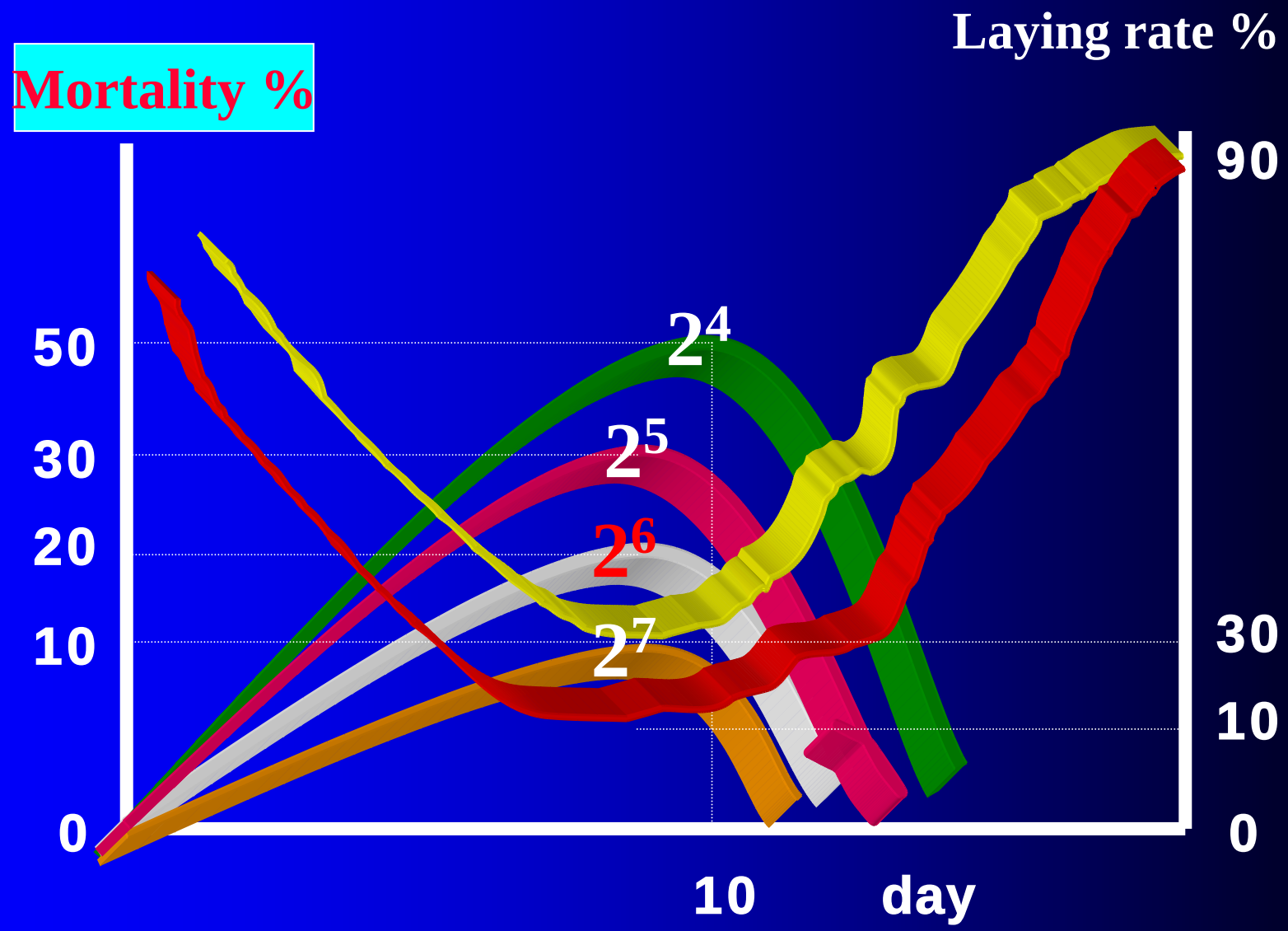
Chicken-Duck mixed raising in opened backyard


virulence
3
2
1
0
-1
-2
-3
1
2
3
4
5
6
Suitable to hosts
passages
Unsuitable to hosts



Correction between environmental virus contamination and antibody protection


Mortality %
Laying rate %
50
30
20
10
0
90
30
10
0
2^4
2^5
2^6
2^7
10
day

3. Immunizing Methods

1) Vaccination routes;
2) Immunizing dosages: depends on body weight;
3) Immunizing Program;
4) Immunizing methods and side reactions. (pre-warming, site, needles, etc.)

4. Husbandry and management

1) Too dense population increases infection transmission chances;
2) Poor ventilation: NH3 increased, macosal barrier destroyed.
3) Frequent stresses: decrease body resistance.

The above factors are inter related.

V. Virus Variation

HPAI(H5N1) has undergone or is undergoing variations

Gene mutations:

HA	Epitode changes	
PB2	627 Glu→Lys	Virus replication enhanced
NS1	92 Asp → Glu	Virus resistance to immune
NA	49-68 20aa lack	Virus replication scope broadened
NS	263-277 15nt lack	Pathogenicity to chickens and mice increased

HPAI(H5N1) has undergone or is undergoing variations

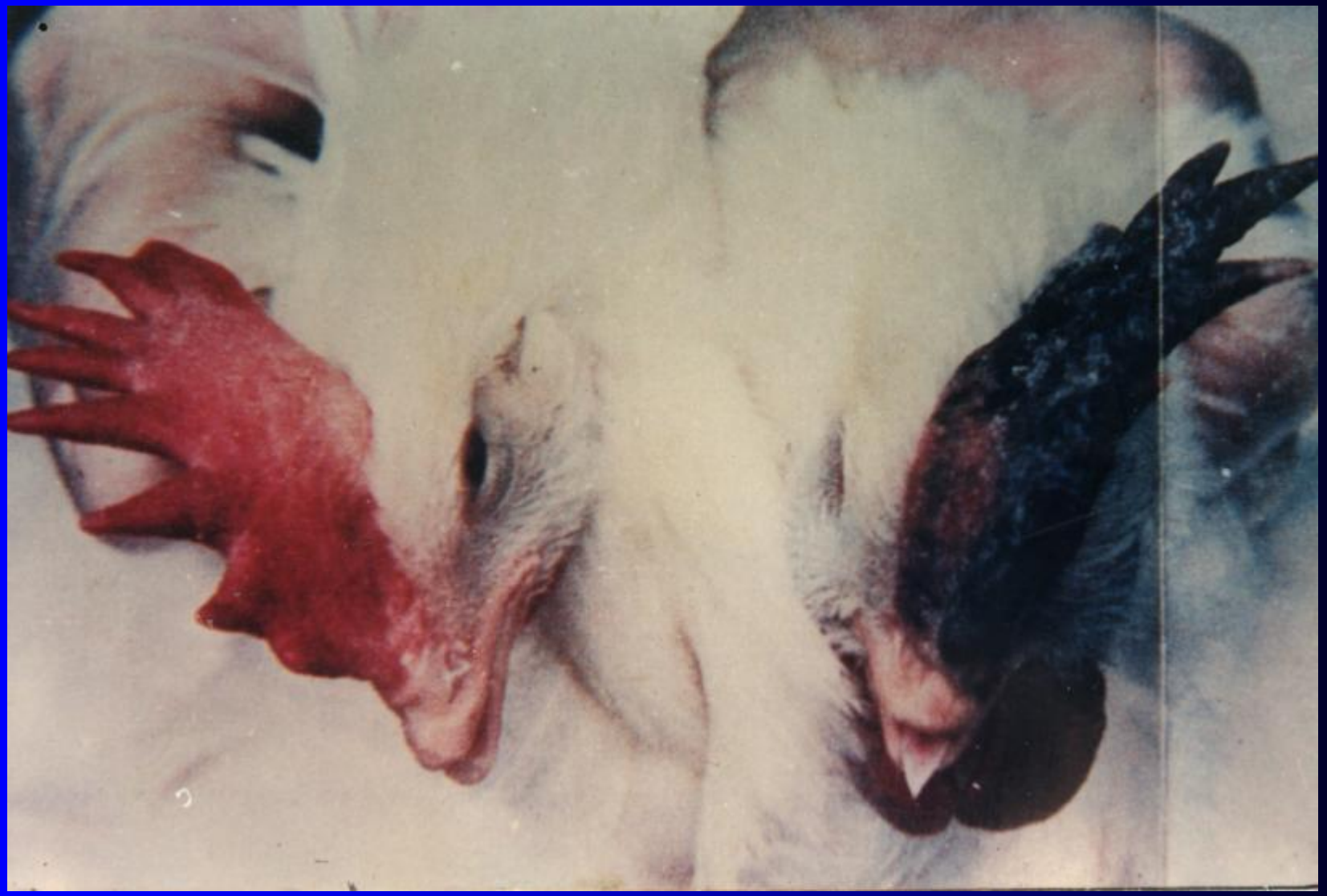
Biological changes:

Avian host range expanded, pathogencity increased, fastal to ducks and migratory birds;

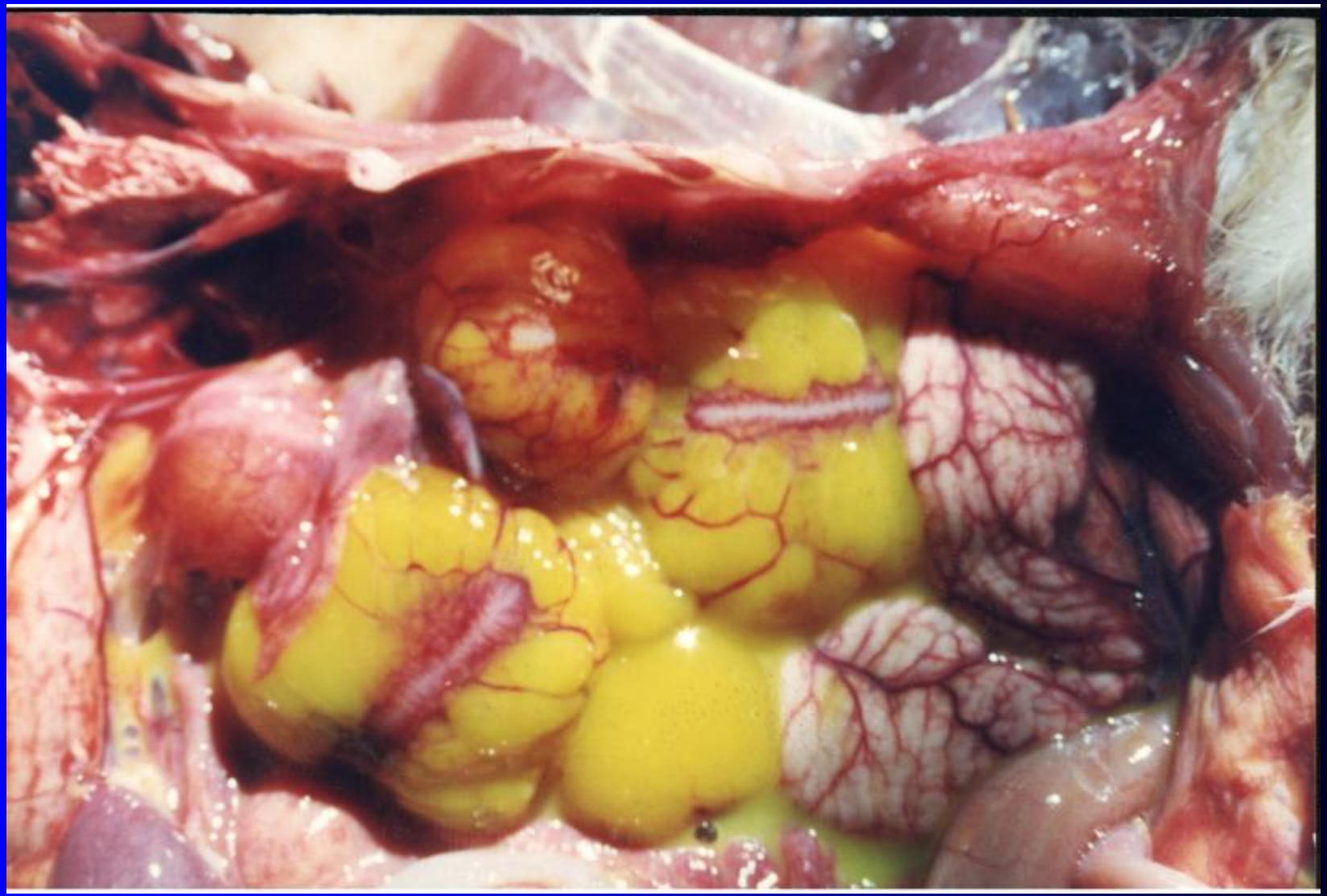
Pathogenicity of mice and ferrets, systematic infection.

Fields such as tiger, lion, leopard and cat may be infected and died.

Resistance to environment increased, transmission routes changed. Aerosol transmission?



AI (H5N1) infected chickens: combs purple blank and swollen; face swollen and cyanosis.



谢谢！

Thank you for your attention!