PROCEEDINGS OF THE JOINT TENTH ANNUAL MEETINGS OF THE NATIONAL NEWCASTLE DISEASE AND AVIAN INFLUENZA LABORATORIES OF COUNTRIES OF THE EUROPEAN UNION

HELD AT THE COMMUNITY REFERENCE LABORATORY, VLA WEYBRIDGE, UK

30th September to 1st OCTOBER 2004

Edited by Dennis J. Alexander

CONTENTS

Contents

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PROGRAMME FOR THURSDAY 30 SEPTEMBER 2004

Annual meeting of the National Laboratories for avian influenza (AI)

9:30 - 9:40	Welcome	
9:40 - 10:00	Technical Report from the EU Reference Laboratory	D. Alexander
10:00 - 10:40.	Current Situation in Italy	G. Cattoli
10:40 - 11:00	*Coffee*	
	Original contributions on AI	
11:00 - 11:40	HPAI H5N2 in Texas	D. Senne
11:40 – 12.00	The effect of vaccination on the transmission of influenza	J. van der Goot
12:00 – 12:20	Comparison of RT-PCR with virus isolation in detection of AIV	K. Smietanka
12:20 – 12:40	Pathogenesis of HPAI H7N7 [The Netherlands 2003 virus] in pigeons infected experimentally	W. Shell
12:40 - 14:00	*Lunch*	
	Original contributions on AI continued	
14:00 – 14:45	Use of DIVA in the control of LP H7N2 in a 3.9 million bird layer flock in Connecticut.	D. Senne
14:45 – 15:15	Outbreaks of H5 and H7 AI in the World 1996 - 2004	D. Alexander
15:15 – 15:45	*Coffee*	
15:45 - 16:05	Survey for AI in poultry and wild birds - update	I. Brown
16.05 - 16:25	Country reports on AI based on questionnaires	D. Alexander
16:25 - 17:00	Discussion, laboratory matters, recommendations etc	

PROGRAMME FOR FRIDAY 1 OCTOBER 2004

Annual meeting of the National Laboratories for Newcastle disease (ND)

9:30 - 9:50	Country reports on ND based on questionnaires	D. Alexander
9:50 - 10:20	Report from the European Commission	M. Pittman
10:20 - 10:40	*Coffee*	
	Original contributions on ND	
10.40 – 11.20	Utilization of the National Animal Health Laboratory Network (NAHLN) in disease outbreaks	D. Senne
11.20 – 11.40	Immunoselection and characterisation of attenuated ND virus strains suitable for in ovo vaccination.	T. van den Berg
11.40 - 12.00	Pathogenicity of NDV strains isolated from pigeons in Poland	K. Smietanka
12.00 – 12.20	Selection of different pathotypes from a single isolate of PPMV1	C. Fuller
12.20 - 12.40	PPMV-1 viruses with low pathogenicity indices	D. Graham
12.40 - 13:40	*Lunch*	
13:40 - 14:00	ND situation worldwide excluding EU and USA	R. Manvell
14:00 - 14:20	Interlaboratory comparative tests	D. Alexander
14:20 - 14:35	Work plan of the Community Reference Laboratory for 2005	M. Pittman
14:35 - 15.00	Discussion, laboratory matters, recommendations etc and close	

Annual Meeting of the National Laboratories for Avian Influenza

TECHNICAL REPORT FOR THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2003

I. LEGAL FUNCTIONS AND DUTIES

The functions and duties are specified in Annex V of Council Directive 92/40/EC introducing Community measures for the control of avian influenza (Official Journal of the Communities No L 167 of 22.6.1992).

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2003

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decision 95/233/EC (Official Journal of the European Communities No L 156, p. 76) as amended by Decision 96/619/EC (OJ No L 276, p. 18). This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:

   a) Determining the intravenous pathogenicity index (IVPI)

   b) Antigenic typing of viruses and both haemagglutinin and neuraminidase subtypes

   c) Determining the amino acid sequence at the haemagglutinin cleavage site of H5 and H7 subtype viruses

   d) Limited phylogenetic analysis to assist in epidemiological investigations.

***Work Plan**: The number of viruses received will be dependent on the outbreaks occurring and those viruses submitted, as a guide the numbers received since 1988 are shown in Table 1.*

Table 1. Number of viruses submitted to the CRL each year since 1988

1988	1989	1990	1991	1992	1993	1994	1995
401	188	113	154	199	294	385	605
1996	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**
284	227	285	357	704	316	333	464

The haemagglutinin and neuraminidase subtypes of all influenza viruses submitted will be determined. IVPI tests will be done at the request of the submitting laboratory or the Commission. The amino acids at the haemagglutinin cleavage site of all viruses of H5 and H7 subtype will be deduced by nucleotide sequencing. For selected viruses sequencing will be extended into other areas of the H gene to allow phylogenetic analyses.

***% Resources**: 62 %*

WORK DONE: The viruses submitted in 2003 were characterised as shown in Table 2.

Table 2: Identification of viruses submitted to the CRL in 2003

Virus identification	Number
Paramyxoviruses	**166**
Influenza A viruses	**155**
H1N1	2
H2N3	2
H5N2	1
H5N6	1
H5N7	1
H6N2	5
H7N3	108
H7N7	11
H9N2	21
H10N5	1
H16N3?	2
not yet typed	**112**
virus not viable	**31**

In addition to conventional typing of the viruses submitted 10 representative H5 and H7 viruses were subjected to nucleotide sequencing and the amino acids at the haemagglutinin cleavage site deduced.

Eight intravenous pathogenicity index tests were done at the request of the submitting country on the submitted viruses to assess their virulence.

Estimated actual resources: 64%

2. Maintain and distribute virus repository and reagents necessary for virus characterisation.

Work Plan*: Maintenance of existing repository will continue. All viruses submitted to the CRL will be added to the repository after characterisation. Most viruses will be maintained in a frozen state, but selected, representative viruses will be freeze dried. Reagents such as polyclonal chicken antisera, and control antigens will be maintained at levels previous demands have indicated to be necessary to enable characterisation of all 15 H and all 9 N subtypes.*

% Resources*: 8 %*

WORK DONE: The AI viruses received were added to the repository. Reagent stocks were maintained, at least at previous levels [Table 3] and during the year the following were supplied:

ANTIGENS: 30 x 1ml ampoules of influenza A agar gel precipitin antigen, 4 x 1.0ml of Eq/Prague antigen, 1.0ml of H1 Ag, 172 x 1ml of H5 antigen, 1ml of H6 antigen, 161 x 1ml of H7 antigen, 1ml of H9 antigen.

ANTISERA: 12 x 0.5ml ampoules of H1 serum, 6 x 0.5ml of H2 serum, 10 x 0.5ml of H3 serum, 4 x 0.5ml of H4 serum, 180 x 0.5ml of H5 serum, 8 x 0.5ml of H6 serum, 108 x 0.5ml of H7 serum, 8 x 0.5ml of H8 serum, 14 x 0.5ml of H9 serum, 6 x 0.5ml of H10 serum, 6 x 0.5ml of H11 serum, 8 x 0.5ml of H12 serum, 6 x 0.5ml of H13 serum, 6 x 0.5ml of H14 serum and 8 x 0.5ml of H15 serum. 26 x 1ml of AGP +ve cont serum.

32 x 0.5ml ampoules of SPF chicken serum were also supplied.

Estimated actual % resources**: **8%

Table 3. Stocks of polyclonal chicken sera and virus antigens for HI tests held at the Reference Laboratory.

Type	Serum		Antigen	
	Quantity[a]	HI titre[b]	Quantity[a]	HA titre[b]
SPF	100	<1		
H5	100	7	50	7
H7	250	6	200	7

[a] *Number of freeze-dried ampoules containing 0.5 ml of serum or antigen at the indicated titre.*

[b] *HI and HA titres are expressed as $\log_2$. The SPF serum had an HI titre of <1 to each antigen.*

3. Prepare and distribute antisera, antigens and reagents for the inter-laboratory comparison tests.

***Work Plan**: Antisera and antigens to be used in the comparison tests will be prepared, freeze-dried and dispatched to the National Laboratories in time for results to be reported at the next annual meeting.*

***% Resources**: 6 %*

WORK DONE: Antigens were prepared and dispatched to EU National Laboratories and those of accession countries [total 31 laboratories]

Estimated actual % resources**: **4%

4. Analysis of results submitted by National Laboratories for the inter-laboratory comparison tests.

***Work Plan**: As in previous years, results submitted by the National Laboratories will be analysed and presented at the annual meeting.*

***% Resources**: 3 %*

WORK DONE: *Results were received, analysed and an oral presentation made at the Annual Meeting in 2003. A written report will appear in the proceedings.*

Estimated actual % resources**: **3%

5. Conduct work to evaluate reported problem areas in diagnosis.

***Work Plan**: Staff of the CRL will be available for consultation by National Laboratories, problem sera and other reagents will be received from National Laboratories for testing and evaluation.*

***% Resources:** 2 %*

WORK DONE: Staff of the CRL were consulted on an ad hoc basis.

Estimated actual % resources**: **2%

6. Supporting by means of information and technical advice National Avian Influenza Laboratories and the European Commission during epidemics.

***Work Plan**: Staff of the CRL will be available for consultation and will forward all relevant information to the National Laboratories or the Commission, as appropriate.*

***% Resources:** 2 %*

WORK DONE: Staff of the CRL were consulted on numerous occasions by other National Laboratories representatives of member states and the Commission.

Estimated actual % resources**: **2%

7. Prepare programme and working documents for the Annual Meeting of National Avian Influenza Laboratories.

***Work Plan**: The organisation of the Annual Meeting in collaboration with the Commission's representative will be done as in previous years.*

***% Resources:** 2 %*

WORK DONE: In collaboration with the Commission's representatives the Annual Meeting was organised and held in Brussels in December 2003.

Estimated actual % resources**: **2%

8. Collecting and editing of material for a report covering the annual meeting of National Avian Influenza Laboratories.

***Work Plan**: Receive and collate submissions edit and produce report of 2002 proceedings before 2003 Annual meeting. Receive and collate submissions of 2003 meeting.*

***% Resources**: 3 %*

WORK DONE: Proceedings of the 2002 meeting were produced before the 2003 meeting.

Estimated actual % resources**: **4%

9. In the light of the occurrence of influenza in birds and other animals keep under review the possible zoonotic impact arising from the risk of reassortment between influenza viruses.

***Work Plan**: Analyse data as it becomes available*

***% Resources:** 3%*

WORK DONE: This was done through CRL staff membership of the WHO Animal Influenza Network [1 meeting] and the European Surveillance Network for influenza in pigs [3 meetings]. In addition close watch was kept on situations relating to spread of AI viruses from birds to humans – see publications.

Estimated actual % resources**: **2%

10. Continuation and finalisation of work carried out in respect to the surveys in poultry and wild birds started in 2002.

***Work Plan**: Scientific input into steering surveillance programme through SCFCAW and reviewing proposed national surveillance programmes.*

***% Resources:** 8%*

WORK DONE: The programme went through SCFCAW and the national programmes were reviewed. In addition CRL staff were responsible for the assembly, collation and presentation of data relating to programmes in all member states. Report of the surveillance exercise was written and submitted.

Estimated actual % resources**: **7%

11. Preparation and publications of articles and reports associated with above work.

% Resources: *1%*

WORK DONE:

RELEVANT PUBLICATIONS IN 2003

1. ALEXANDER, D.J. & MANVELL, R.J. (2003). CRL Technical Report for AI 2001. Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Padova, 2002 pp8-13.
2. ALEXANDER, D.J. & MANVELL, R.J. (2003). Country Reports on AI based on questionnaires Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Padova, 2002 pp 14-33.
3. ALEXANDER, D.J. & MANVELL, R.J. (2003). Interlaboratory comparative tests. Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Padova, 2002 pp 94-99.
4. BROWN, I.H. (2003) Surveillance for AI in poultry and wild birds. Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Padova, 2002 pp 54-63.
5. ALEXANDER, D.J. (2003). Report on avian influenza in the Eastern Hemisphere during 1997-2002. Proceedings of the 5th International Symposium on Avian Influenza, Athens, Georgia, April 14-17 2002. Avian Diseases 47, 792-797.
6. ALEXANDER, D.J. (2003). Should we change the definition of avian influenza for eradication purposes? Proceedings of the 5th International Symposium on Avian Influenza, Athens, Georgia, April 14-17 2002. Avian Diseases 47, 976-981.
7. ALEXANDER, D.J. (2003) Influenza aviar – Enfermedad y Diagnostico [Avian Influenza Disease and Diagnosis] In: *Ponencias de XL Symposium Cientifico De Avicultura of the Spanish WPSA Branch Girona 2-3 October 2003.* pp 111-117
8. ALEXANDER, D.J. (2003). Avian influenza – General overview and current situation in Europe Abstracts of “Strategie di difesa del comparto avicolo: il punto della situazione sull' emergenza dell' influenza aviare”. Forli, Italy June 2003.
9. CAPUA, I. & ALEXANDER D.J. (2003). The proposed new OIE chapter on avian influenza. Abstracts of II Seminarion Internacional Influenza Aviar y Enfermedad de Newcastle Lima, Peru August 2003.
10. CAPUA, I. & ALEXANDER, D.J. (2003) An update on avian influenza control. Proceedings of the 6th International Congress of veterinary Virology ESVV. St Malo August 2003 p48.
11. ALEXANDER, D.J. (2003) Avian influenza as a zoonosis. Abstracts of IBMS Biomedical Science Congress Birmingham September 2003 p43.

12. CAPUA, I. & ALEXANDER D.J. (2003). Recent developments on avian influenza. Abstracts of Options for the control of influenza V. Okinawa, Japan. W09P-05.
13. SUAREZ, D.L. SENNE, D.A., BANKS, J., BROWN, I.H., ESSEN, S.C., LEE, C.W., MANVELL, R.J., MATHIEU-BENSON, C., PEDERSEN, J., PANIGRAHY, B., SPACKMAN, E. & ALEXANDER, D.J. (2003). A shift in virulence in the influenza A subtype H7N3 virus responsible for a natural outbreak of avian influenza in Chile appears to be the result of recombination. Abstracts Options for the control of influenza V. Okinawa, Japan. W09-04.
14. ALEXANDER, D.J. (2003). Influenza. Abstracts of British Ornithologists' Union Seminar on Birds and Public Health November 2003 12-13.
15. MANVELL, R., ENGLISH, C., JORGENSEN, P., & BROWN I. (2003) Pathogenesis of H7 influenza A viruses isolated from Ostriches in the homologous host infected experimentally. Proceedings of Fifth International Symposium on Avian Influenza, Athens, Georgia, USA
16. BANKS, J. & PLOWRIGHT L. (2003) Additional Glycosylation at the Receptor Binding Site of the Hemagglutinin (HA) for H5 and H7 Viruses may be an Adaptation to Poultry Hosts, but does it Influence Pathogenicity? 5th International Symposium on avian influenza Athens, Georgia, USA.
17. FOUCHIER, R.A.M., OSTERHAUS, A.D.M.E. & BROWN, I.H. (2003). Animal influenza virus surveillance. Vaccine 21, 1754-57.

Estimated actual % resources**: **2%

It is understood that the above mentioned objectives are not exclusive to other work of more immediate priority which may arise during the given period.

AN UPDATE ON AVIAN INFLUENZA BETWEEN 2003 AND 2004 IN ITALY

Giovanni Cattoli[1], Manuela dalla Pozza[2], Stefano Marangon[2] & Ilaria Capua[1]

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Introduction

During the month of August 2002, serological positivity at the abattoir to an H7 virus was detected in 3 meat turkey flocks in Brescia province (Lombardia region). Intensive surveillance in the whole area did not allow the identification of additional outbreaks. In October 2002, haemagglutination inhibition (HI) tests on serum samples from meat turkeys in the Brescia province were again found to be positive for antibodies to the H7 subtype of avian influenza.

An influenza A virus of the H7N3 subtype was isolated from specimens collected in the seropositive meat turkey flock. The virulence assays performed indicated that the isolate was of low pathogenicity. The intravenous pathogenicity index was 0.0 and the deduced sequence of the cleavage site of the haemagglutinin molecule was of PEIPKGR*GLF and thus did not contain multiple basic amino acids, which are considered a marker for virulence.

Phylogenetic analysis performed on the haemagglutinin (H) gene indicated that this isolate is part of the Eurasian lineage of H7 viruses. The virus was related, but not identical, to the H7N1 virus that caused the 1999-2001 avian influenza epidemic in Italy. The virus was also unrelated to the H7N3 strain contained in the inactivated vaccine (A/ck/Pakistan/95) used in the 2000-2002 vaccination campaign (6). Sequence data obtained from early isolates indicate the presence of a neuraminidase stalk deletion and the absence of additional glycosilation sites at the globular head of the haemagglutinin molecule, which are considered a result of acquired adaptation to the domestic host (7).

Management of the epidemic during 2003

The H7N3 LPAI strain rapidly spread among poultry flocks located in the densely populated poultry area (DPPA) which had been affected by the H7N1 epidemic in 1999-2001, for this reason a vaccination programme was prepared, approved by the EC Commission and enforced to support the other eradication measures in force (stamping out and control marketing of infected flocks, restriction policies to restocking and to movement of live birds, vehicles and staff, intensive monitoring programs). The vaccination strategy proposed and applied was that of using an inactivated oil emulsion vaccine containing a strain with a homologous haemagglutinin (H) group and a heterologous neuraminidase (N) group. The reason for this was the possibility of using it as a natural "marker" vaccine, or more correctly a DIVA [Differentiating Infected from Vaccinated Animals] vaccine (2). The vaccination programme was carried out using an AI inactivated heterologous vaccine (strain A/ck/IT/1999-H7N1). The beginning of the DIVA vaccination campaign was delayed up to the 31st of December 2002, due to unavailability of an appropriate vaccine. From October

the 10th 2002 to 30th of September 2003, the H7N3 LPAI virus was able to spread and infect a total of 388 poultry holdings: 332 meat-type turkey, 5 turkey breeder, 12 broiler breeder, 13 layer, 6 guinea fowl, 4 broiler, 3 quail, 1 meat duck farms and 11 back-yard flocks mainly located in the southern part of the two Italian regions. A total of 7,659,303 birds were involved in the epidemic, and among these 4,230,750 animals were stamped out in 163 affected flocks. The remaining 3,428,553 slaughterbirds were subjected to controlled marketing. Of the affected farms, 88 were vaccinated turkey flocks. The first outbreak in a vaccinated flock occurred on the 18th of April. All the infected vaccinated flocks were meat turkeys mainly located in a limited area of the southern part of Verona province, with the highest concentration of turkey holdings in the country. It is interesting to point out that despite the poultry density in the latter area only 2 unvaccinated poultry farms (1 broiler breeder and 1 meat duck farms) were affected. These farms were located in close proximity to previously vaccinated meat turkey farms which had been field exposed. Stamping out measures or controlled marketing were enforced in all infected flocks which housed a total of 1,523,320 birds. The last infected flock was stamped out on the 9th of October 2003.

A novel vaccination strategy was adopted in 2004

Subsequently to the stamping out of the last infected flock, a monitoring program was applied in the area in order to reveal the circulation of any influenza viruses in the domestic poultry farms. the program was based on serology and tracheal and cloacal swabs for virus detection.

In February 2004, a low pathogenic avian influenza virus strain of subtype H5N3 was isolated in one duck flock in the region of Lombardia within the vaccination area. Although the epidemiological investigations have not revealed any spread of the infection, the risk of introduction of avian influenza subtype H5 was demonstrated.

Therefore, Italy requested to amend the current vaccination programme in order to authorise the vaccination of poultry within the established vaccination area with a bivalent vaccine which protects against avian influenza virus infection of both H7 and H5 subtypes.

The vaccination programme was approved by the EU Commission (decision 666/2004/EC) and it started on 1st October 2004. The type of vaccine is a bivalent, inactivated vaccine containing the strains A/ck/Italy/22a/98 (H5N9) and A/ck/Italy/1067/99 (H7N1). the estimated duration of this vaccination program is until 31st December 2005.

The new bivalent vaccine scheme basically includes two vaccinations in meat turkeys and turkey breeders, capons, chicken and guinea fowl breeders and table eggs layers.

With the exception of the H5N3 isolate in the duck flock, no evidence of virus circulation was revealed from September 2003 to August 2004 in the monitored poultry population (vaccinated and not vaccinated).

During the ongoing monitoring program, on the 15th September 2004, a seropositivity for H7 was detected in a meat turkey flock in the Verona province. Brds of that flock were vaccinated only once. Serology revealed the spreading of the infection in 3 additional vaccinated turkey flocks located in the same municipality. On the 20th September a low pathogenic avian influenza virus strain of subtype H7N3 was isolated in one of these flocks.

Based on the sequencing data, the haemagglutinin molecule was genetically related to the previous H7N3 Italian epidemic strain with a nucleotide homology up to 99.3 %. In addition, the presence of stalk deletion in the NA molecule and of potential additional glicosylation sites (in position 149) in the HA molecule indicated a certain degree of adaptation to the domestic host. Therefore, genetic data suggested that this isolate could be considered as a re-emergence of the H7N3 viruses previously circulating in the area.

Discussion

The analysis of the data gathered during 2003 and 2004 indicates that North-eastern Italy can definitely be considered as an area "at risk" for avian influenza infections. This is not only supported by AI epidemics which have occurred in the past (1,9,10,11,12,13) caused by viruses of the H6, H9 and H7 subtypes, but also by the recent description of an H5 subtype in domestic ducks. This could probably be related to the great numbers of wild birds which fly over the area during their migration, to the great numbers of imports of live birds into the area and to the existence of an undetected link between the reservoir of the infection and the domestic bird populations. For this reason, and considering the poultry density in the area, it is imperative that surveillance programs are implemented to diagnose AI infections promptly.

Vaccination performed in a framework of a DIVA strategy do not mask the infection and do not interfere with the control and the eradication of the disease. In this regard, our findings did demonstrate that an appropriate surveillance program combined with an *ad hoc* vaccination strategy was capable of identifying viral circulation within the vaccinated population rapidly.

It is a point of discussion whether or not the surveillance program should be restricted to industrial poultry farms, as it has been so far.

The re-emergence of a domestic host-adapted H7N3 virus approximately after 11 months of its presumed eradication suggests the existence in the area of niches in which the virus is able to persist. It is likely, from the follow up investigations that a large quail operation could have harboured the virus in the absence of any clinical or serological indication of infection.

In our opinion, the surveillance program should therefore be improved, aiming at the identification of undetected sources of infection.

The control of LPAI infections in DPPA is a challenging experience. The experience gathered during the Italian 1997-2004 AI epidemics suggests that countries at risk of infection should have contingency plans and a general preparedness in order to deal appropriately with such infections. Outbreaks caused by avian influenza viruses of the H5 and H7 subtypes can no longer be considered rare events and therefore alternative strategies to a stamping out policy should be considered, particularly for outbreaks occurring in densely populated poultry areas.

Acknowledgments

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Sperimentale delle Venezie.

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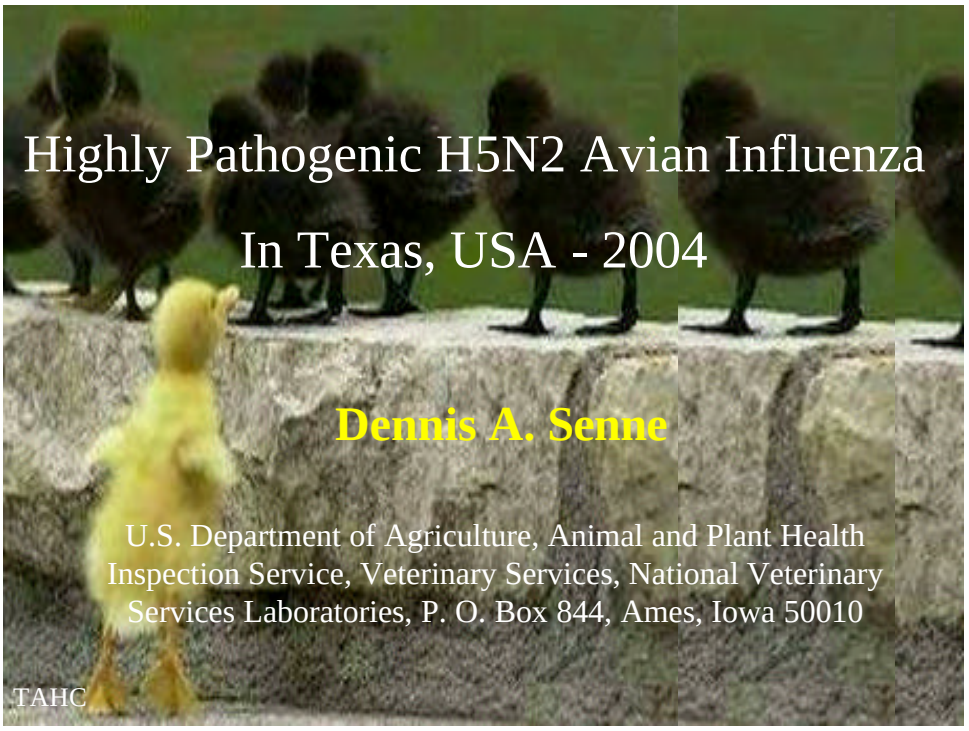
Highly Pathogenic H5N2 Avian Influenza
In Texas, USA - 2004
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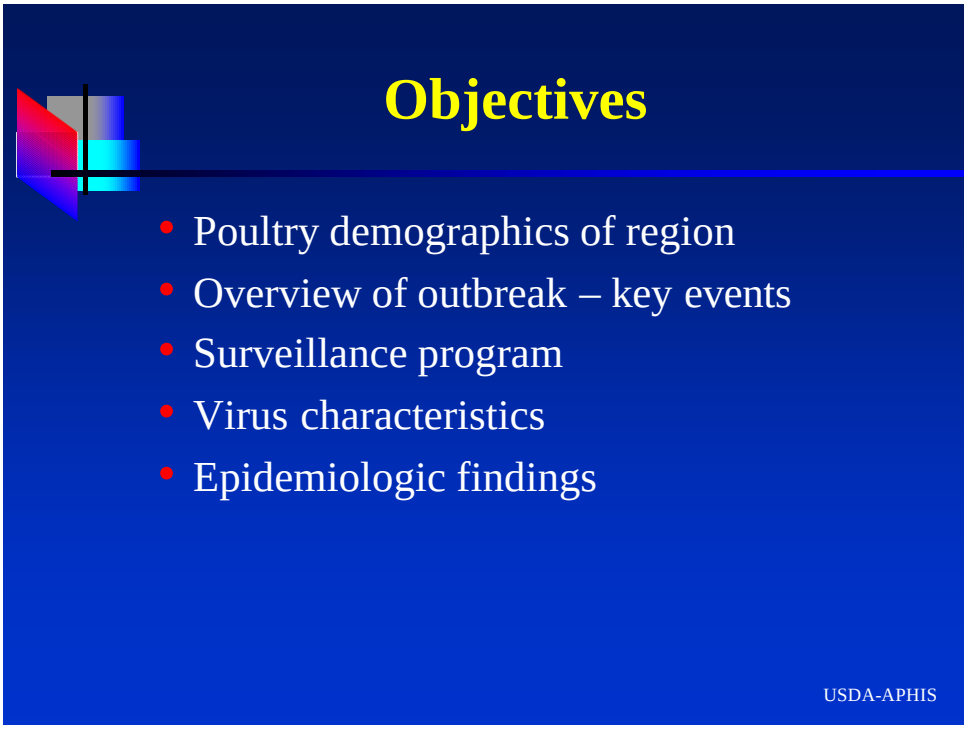
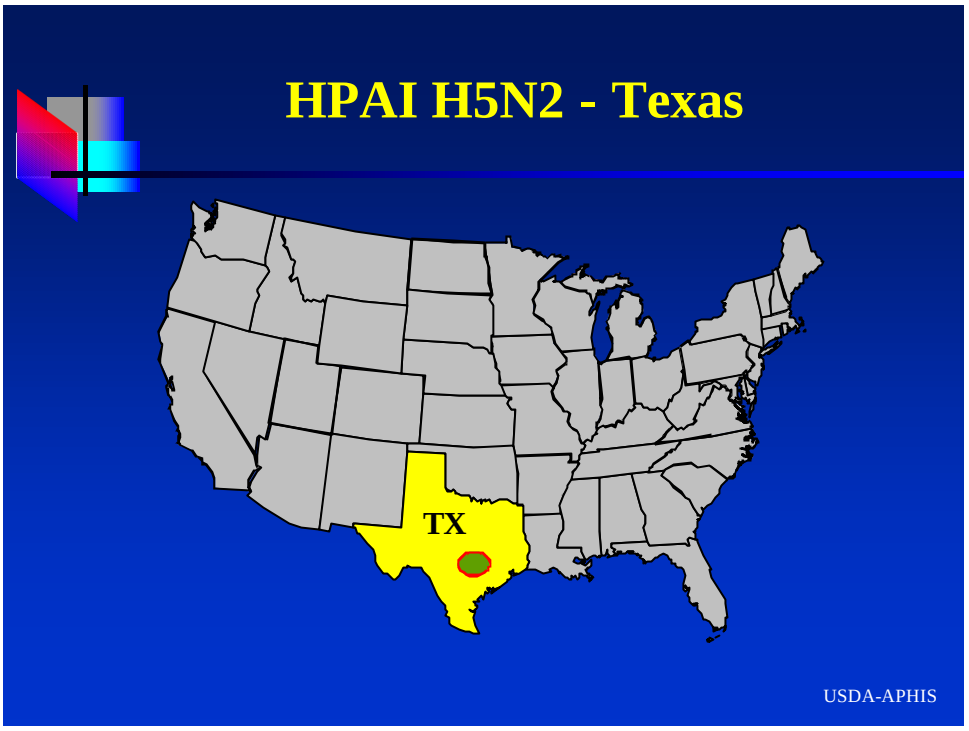
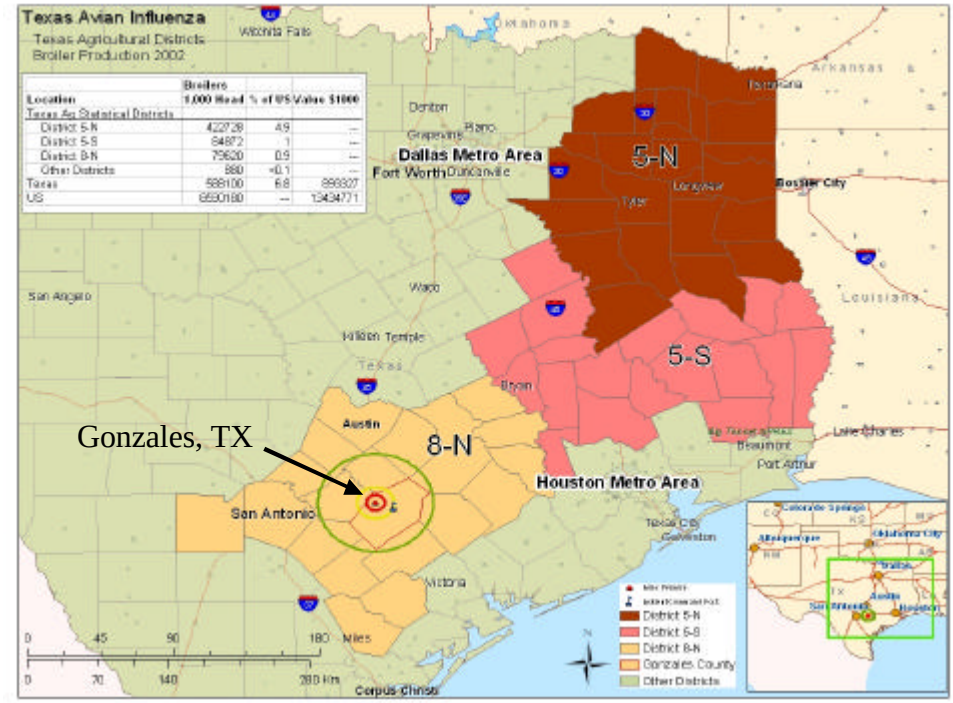
U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, P. O. Box 844, Ames, Iowa 50010
TAHC

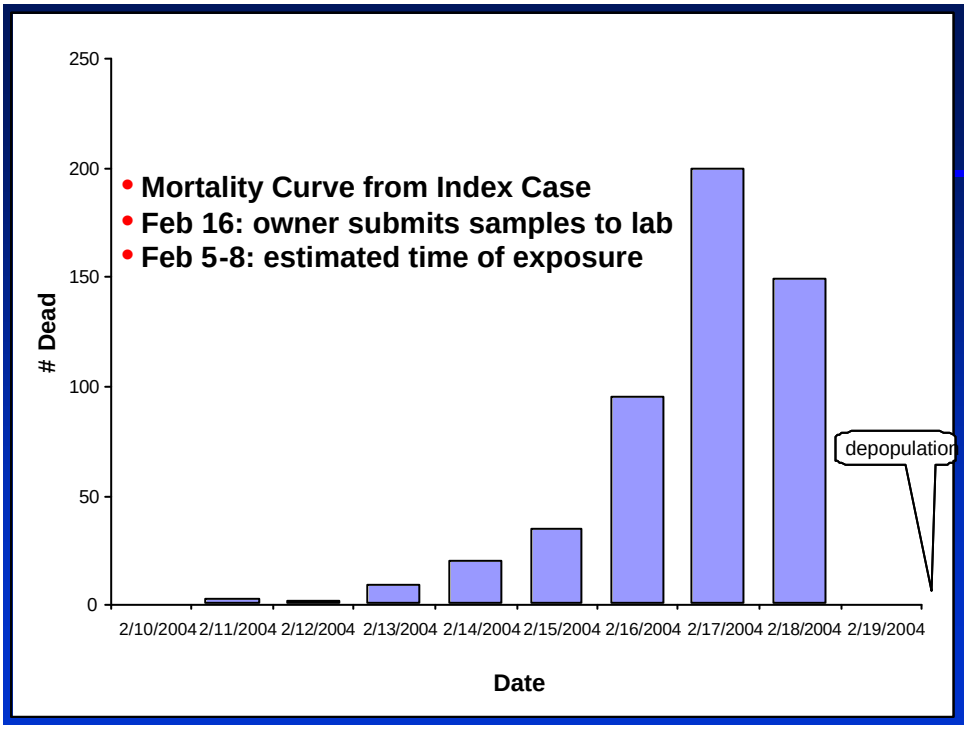

Objectives
Poultry demographics of region
Overview of outbreak – key events
Surveillance program
Virus characteristics
Epidemiologic findings
USDA-APHIS


HPAI H5N2 - Texas
TX
USDA-APHIS


Texas Avian Influenza
Texas Agricultural Districts
Broiler Production 2002
Location
Broilers
1,000 Head
% of US
Value $1000
Texas Ag Statistical Districts
District 5-N
District 5-S
District 8-N
Other Districts
Texas
US
Dallas Metro Area
Fort Worth
5-N
5-S
8-N
Houston Metro Area
Gonzales, TX
San Antonio
Austin
Waco
Victoria
Corpus Christi
Lake Charles
Port Arthur
Beaumont
Louisiana
Arkansas
Oklahoma
Wichita Falls
San Angelo
Killeen-Temple
Texas
Byron
Denton
Plano
Grapevine
Duncanville
Longview
Tyler
Bossier City
Texas City
Galveston
Gonzales County
Other Districts
0 45 90 180 Miles
0 70 140 280 Km

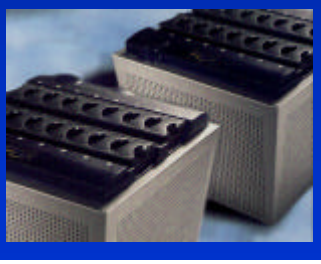
HPAI H5N2 – TX

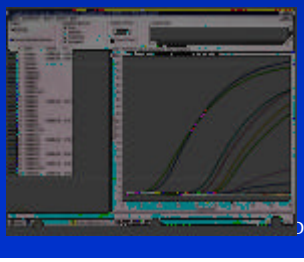
- Feb 16 – owner notes increased mortality non-commercial broiler flock in Gonzales, TX
  - ✓6,600 birds
  - ✓Respiratory signs (suggestive of MG)



HPAI H5N2 – TX

- Feb 17 – TX Vet Med Diag Lab
  - ✓Serum positive AGID
  - ✓Swabs positive RRT-PCR for H5
- Samples to NVSL for confirmation





Feb 19…

- Epi-connection with LBMs in Houston, TX
- Hold Order placed on all five LBMs (and their associated holding facilities) in Houston and samples collected (serum and swabs)

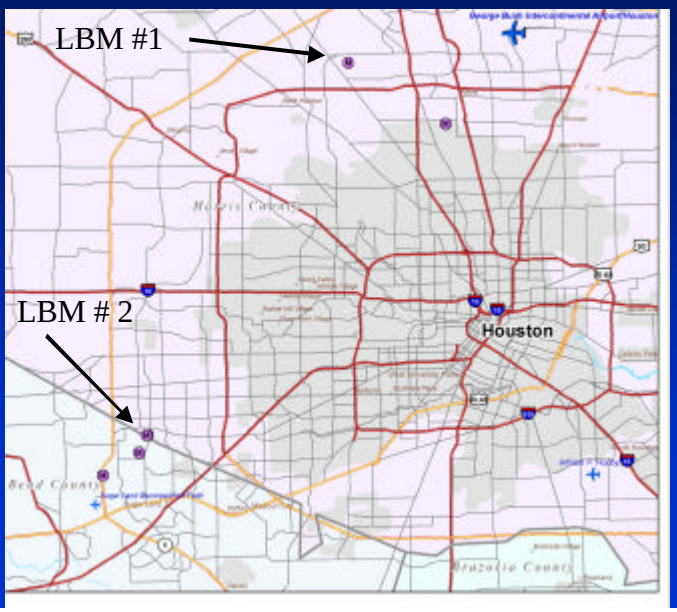
Feb 20…
NVSL confirms H5N2 Avian Influenza

Feb 21…
Euthanasia and disposal of index flock- (6,608 birds)

Feb 22…

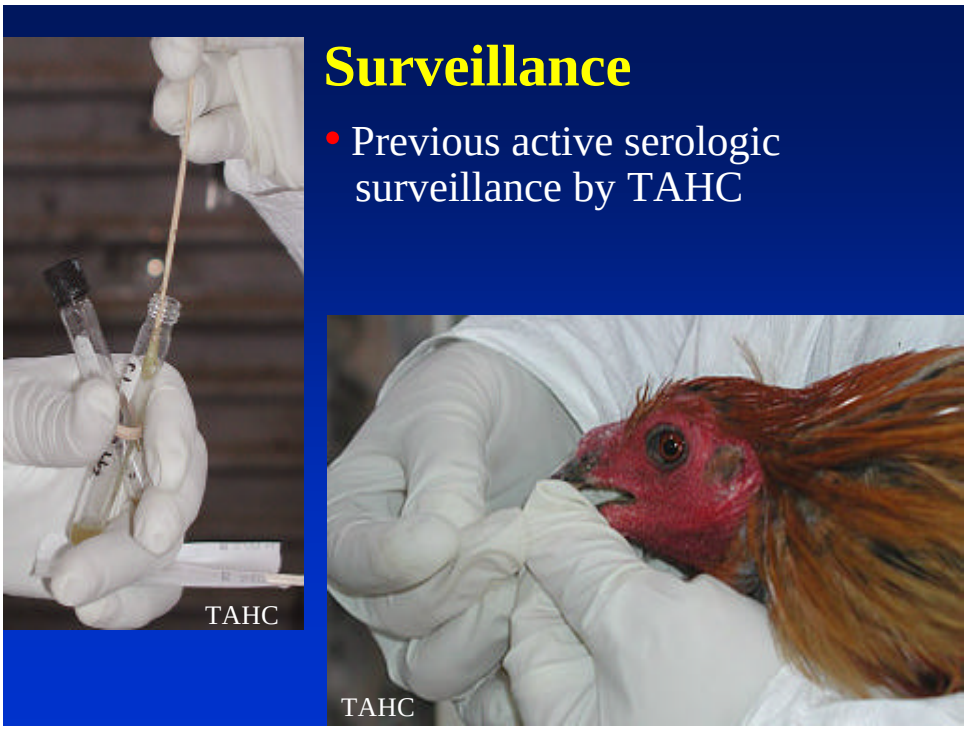
- H5N2 AI confirmed in two LBMs (VI)
- Positive LBMs quarantined

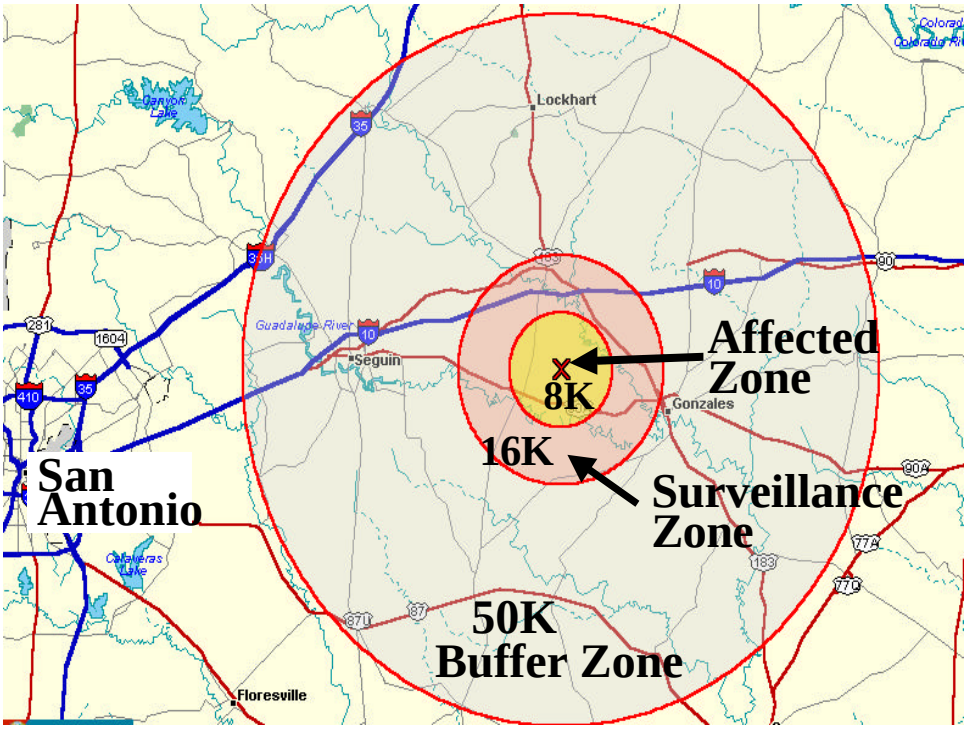
Two infected LBMs in Houston suburbs



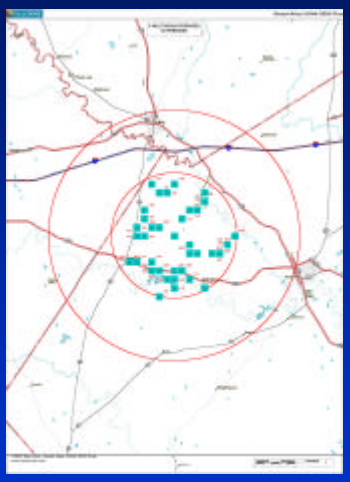
Feb 23…

- HPAI confirmed and reported to OIE
  - ✓Virus meets molecular criteron
  - ✓IVPI = 0.0 (March 1)
- Quarantine placed on index farm and the 2 infected LBMs
- USDA established joint Incident Command Post with TAHC in Gonzales, TX

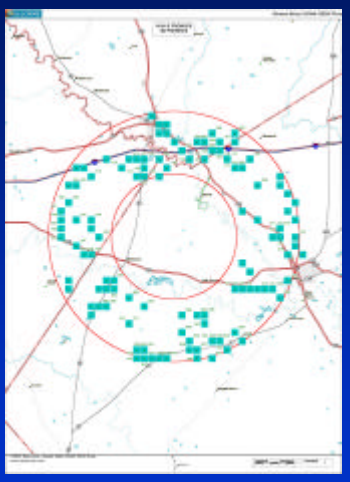

Surveillance
• Previous active serologic surveillance by TAHC
TAHC
TAHC


Lockhart
Seguin
Gonzales
Floresville
San Antonio
Affected Zone
8K
16K
Surveillance Zone
50K Buffer Zone

HPAI H5N2 – Gonzales, Texas



8 K "infected zone"
39 non-commercial
5 commercial



16 K "surveillance zone"
178 non-commercial
35 commercial

4-Week Surveillance Plan sampling schedule

	Week 1 3/1-3/7		Week 2 3/8-3/14		Week 3 3/15-3/21		Week 4 3/22-3/28	
	Serum	-swab	Serum	-swab	Serum	-swab	Serum	-swab
Affected Zone	Y	Y	N	Y	N	Y	N	Y
Surv. Zone	Y	Y	N	N	N	N	N	Y
Buffer Zone	N	N	N	N	Swab only			

Note: This plan was initiated after the baseline sampling was completed

Sampling (Tracheal Swabs)
CI = 95/25

Number of birds on premise or in House	Minimum number to be sampled
10 or less	Sample all
20	15
30	15
40	15
50 or more	20 per house

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Laboratory Surveillance & Monitoring
Summary

	Serology	Swabs/PCR
Task Force Surveillance	**4,262**	**4,051**
TX Poultry Federation routine monitoring (Dec, Jan, Feb) Gonzales area	**11,947**	
TX Poultry Federation routine monitoring (Dec, Jan, Feb) Entire state	**23,039**	

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Virus Characteristics

- Amino acid sequence compatible with HPAI
- **H5N2 - PQRKKR/GLF**
- A/Ck/Scotland/59 **PQRKKR/GLF**
- IVPI = 0.0
- Closely related to A/CK/TX/02 (H5N3)
  - ✓98% sequence homology (HA gene)
  - ✓Two nucleotide changes near cleavage site:

(TX/02) **PQREKR/GLF** → ? (TX/04) **PQRKKR/GLF**

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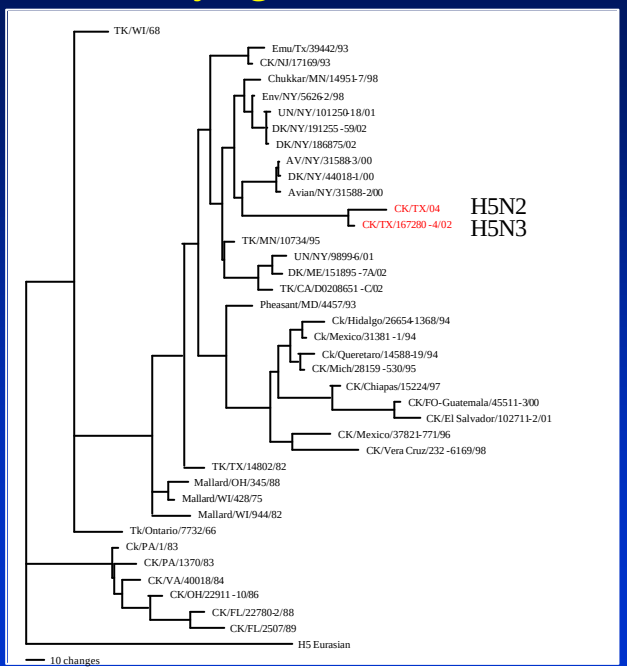
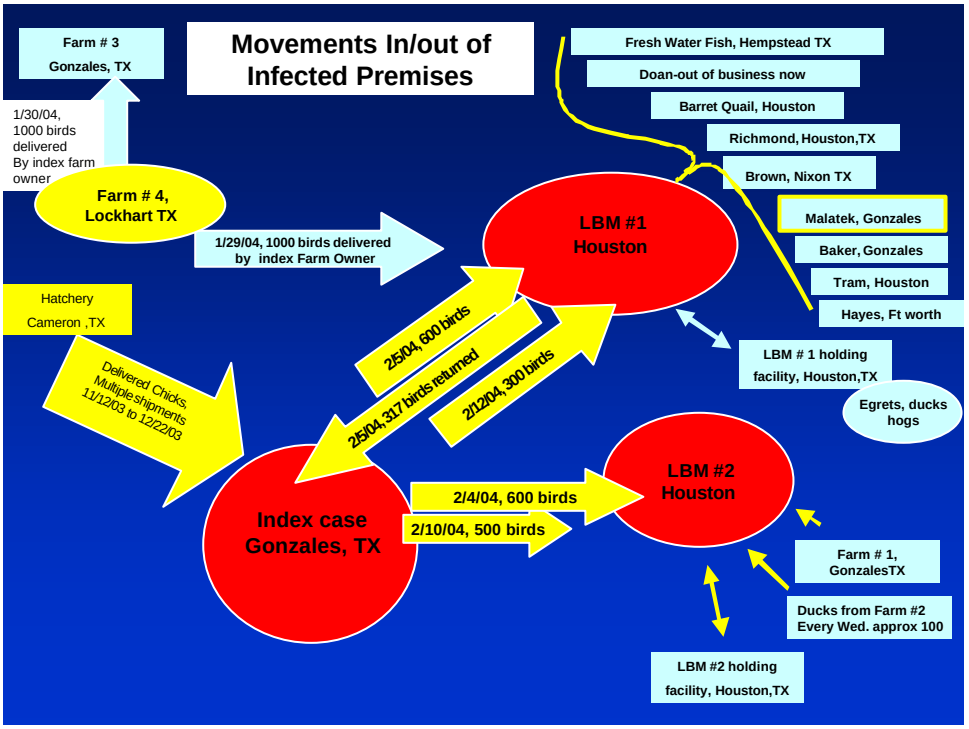
H5 Phylogenetic Tree

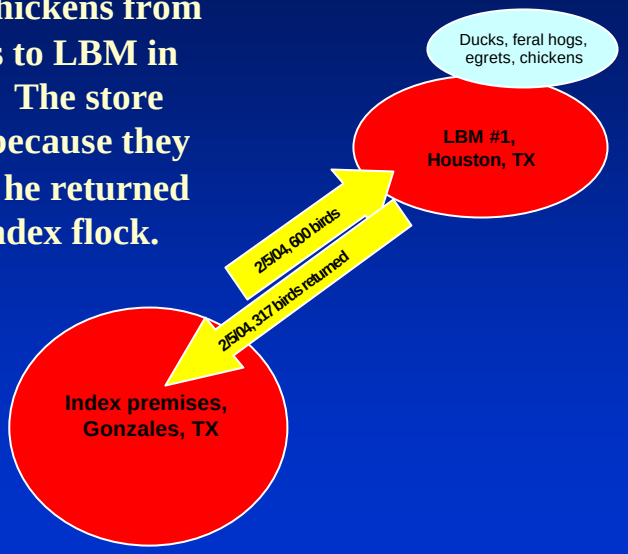


Phylogenetic tree courtesy D. Suarez, SEPRL

Epidemiologic Findings



On 2/5/04 600 chickens from index premises to LBM in Houston, TX. The store rejected some because they were small and he returned them to the index flock.



Live Bird Market #1

- Small facility with high volume of trade (~1,500 per week)
- No clinical signs in the infected hens
- Primarily in a residential and commercial area of Houston

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LBM #1 Holding Pens:

Customers choose birds, slaughtered on site

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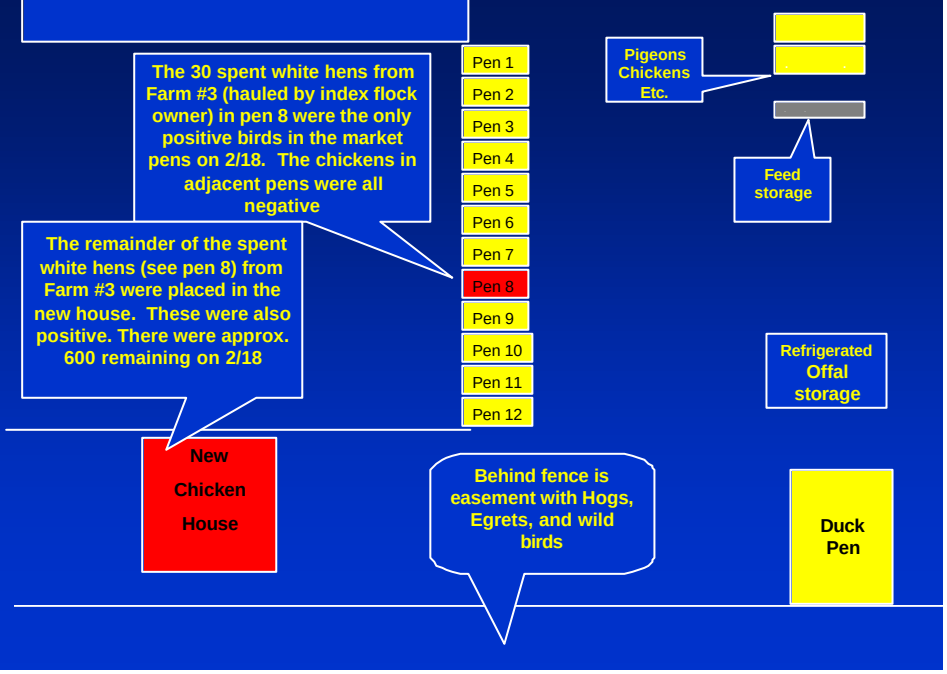
LBM #1 Store Front



"New Chicken House" at LBM #1:

The infected white spent hens were the first birds placed in new house

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LBM #1:

Duck pen located in premises

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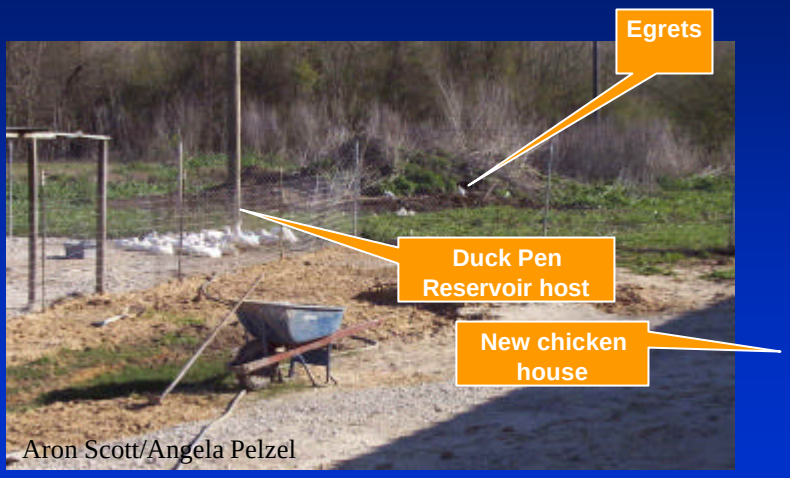
LBM #1:

Egrets feeding on organic material where feral hogs have been rooting



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LBM #1:

Hole in fence allowing feral pigs access to premises (new chicken house and the duck pen)

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Summary

- Small outbreak of HP H5N2 – February 2004
  - ✓One non-commercial broiler facility in Gonzales, TX
  - ✓2 live-bird markets (LBMs) in Houston, TX
- Epi-link between the index case and LBMs
- Virus meets molecular criteria but IVPI = 0.0
- Economic consequences significant
  - ✓Surveillance costs
  - ✓Trade restrictions

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TRANSMISSION EXPERIMENTS TO STUDY THE EFFECT OF VACCINATION ON H7N7 AVIAN INFLUENZA IN POULTRY

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[b]Quantitative Veterinary Epidemiology, Animal Sciences Group, Wageningen University Research, Lelystad

1. INTRODUCTION

Highly Pathogenic Avian influenza (HPAI) is a viral disease of poultry caused by H5 or H7 avian influenza with high morbidity and mortality. Outbreaks of HPAI have a devastating effect on poultry and the poultry industry. Measurements taken in the EU during the most recent outbreaks of HPAI (Italy 1999, Netherlands 2003) were stamping out of infected flocks and pre-emptive culling. There is a possibility of emergency vaccination, but this has never been applied during an outbreak of HPAI. Besides the economic consequences of an emergency vaccination there are other questions about vaccination: it might "mask" the infection, this means that the virus can spread unnoticed in the vaccinated population because it protects the birds from disease and mortality but not from infection.
Inactivated oil emulsion vaccines protect chickens against morbidity and mortality after challenge with HPAI (Capua 2002, Swayne *et al*. 1999, 2000). In most cases there was still shedding of virus from the trachea or cloaca, but compared to non-vaccinated animals there was usually a reduction in the amount of virus shed. What the consequences of this shedding are for the spread of virus in the flock has never been studied or quantified.
We used transmission experiments to study this *within-herd* transmission. These type of experiments have been described previously (De Jong and Kimman, 1994; Van der Goot *et al*. 2003). Advantages of transmission experiments are that it is possible to study one single factor or treatment under controlled conditions *e.g.* different vaccines and vaccination schedules. This would be much more difficult in the field situation due to a lot of variation between animals. In the experiments we compared vaccinated chickens (with two different vaccines) with a non-vaccinated group and we compared the vaccination at two different timepoints before challenge.

2. MATERIALS AND METHODS

2.1 Virus, chickens, vaccines

The influenza virus used in this study was A/Chicken/Netherland/621557/03 H7N7. In all experiments six weeks old SPF white leghorn chickens were used. The chickens were inoculated both intranasaly and intratracheally with 0.1 ml diluted allantoic fluid containing 10^6 median egg infectious dose (EID50) per ml. Two commercially avialable vaccines were used: an inactivated oil emulsion H7N1 vaccine, and an inactivated oil emulsion H7N3 vaccine

2.2 Transmission experiments

Group transmission experiments. Twenty-four SPF White Leghorn chickens (six weeks) were vaccinated. One or two weeks after the vaccination 10 chickens were placed into cages: five animals per cage. They were inoculated with virus and 24 hours later five animals per cage were added. In the same room two other groups of two chickens were placed (air contact sentinels). The animals were monitored by taking tracheal- and cloacal swabs daily during the first 10 days and twice a week for the next 11 days. A blood sample was taken once a week. Three weeks after the challenge the experiment was terminated. The same experiment was performed without vaccination.

Paired transmission experiments. Eight SPF White Leghorn chickens (six weeks) were vaccinated, four with the H7N1 vaccine and four with the H7N3 vaccine. One or two weeks after vaccination all eight animals were challenged with H7N7 virus. After 24 hours one SPF non-vaccinated chicken was added to one infected vaccinated chicken, each pair was housed in a separate cage. The birds were monitored during 14 days by taking tracheal and cloacal swabs daily during the first 10 days, and a last swab at day 14. A blood sample was taken once a week. As soon as a contact bird showed signs of illness the animal was killed.

2.3 Laboratory assays

Virusisolation. Swabs were put in 2 ml 2.95% tryptose phosphate buffer with 5 x 10^3 IU of penicillin-sodium and 5 mg streptomycin per ml. The swabs were stored at -70°C until analysed. Three embryonated chicken eggs incubated for 9 days were inoculated with 0.2 ml per egg. After 72h the allantoic fluid was harvested. A haemagglutination assay (HA) was performed following standard procedures. When at least one of the eggs was positive in the HA assay the swab was considered to be positive.

2.4 Statistical analysis

Statistical analyses: The analysis of the transmission experiments is based on a stochastic SEIR epidemic model in which individuals are either susceptible (S), latently infected (i.e. infected but not yet infectious)(E), infected and infectious (I), and either recovered and immune or else dead (R). The analyses are aimed at estimation of the (basic) reproduction ratio. The reproduction ratio (denoted by R) is defined as the mean number of infections that would be caused by a single infected individual in a large population of susceptible animals. If $R>1$, an infected animal infects on average more than *1* susceptible animal, and a chain reaction of infections may occur. If $R<1$, a prolonged chain reaction of infections is not possible, and the epidemic comes to a halt. In our context, the reproduction ratio is given by the product of the mean infectious period $E(T_I)$ (dimension: *time*) and the transmission rate parameter $ß$(dimension: $time^{-1}$): $R=ßE(T_I)$.

We use two different methods to estimate the reproduction ratio: (*i*) final size methods and (*ii*) a Generalized Linear Model. The appeal of final size methods is that they are flexible and robust (Ball, 1986, 1995; Kroese & De Jong, 2001). For instance, the final size does not depend on whether or not there is a period of latency, and different assumptions on the infectious period distribution are easily incorporated. On the other hand, final size methods do not make use of

all the information, and do not allow separate estimation of the transmission rate parameter and infectious period. For this purpose the Generalized Linear Model is appropriate (Becker, 1989).

3. RESULTS

It was shown that vaccination reduces spread of virus within a flock. The amount of reduction depends on the type of vaccine and the time of challenge after vaccination. When challenged two weeks after vaccination both vaccines were able to prevent all spreading of virus, no virus could be detected from the trachea or cloaca of the challenged or contact animals. When challenged one week after vaccination we were able to detect virus from the trachea and cloaca of the challenged animals and some of the contact animals, with a difference between the two vaccines.

4. DISCUSSION

It was demonstrated that vaccination reduces the *within-herd* transmission. When the R within the flock is below 1 the R between flocks will also be below 1 and this is the aim of vaccination. This reduction in transmission is achieved between one and two weeks after vaccination, and it was demonstrated that after this time there is not a “masked” infection. This means that vaccination can be a valuable tool during an outbreak, but care should be taken when extrapolating these findings to the field situation. In the field there are other factors that may influence the spread of virus: secundary infections, heterogeneity in the immune-response of the animals, housing systems (cages), feed and water systems, climate, *etc*.
For the future it would be interesting to study other species (in this study layer chickens), because during an outbreak more species are involved, for example in the Italian outbreak turkeys played a major role, and in the current outbreaks in Asia ducks appear to be important.

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COMPARISON OF RT-PCR WITH VIRUS ISOLATION FOR THE DETECTION OF AIV

Krzysztof Smietanka, Zenon Minta, Katarzyna Domanska-Blicharz

National Reference Laboratory for Avian Influenza and Newcastle Disease, National Veterinary Research Institute, Pulawy, Poland

A quick and reliable diagnosis of avian influenza (AI) infections is crucial in the control of the disease. Diagnostic methods currently recommended by EU and OIE (2,6) comprise virus isolation on specific pathogen free (SPF) embryonated eggs and identification in haemagglutination inhibition test (HI) followed by assessment of pathogenicity on SPF chickens or, alternatively, reverse-transcription polymerase chain reaction (RT-PCR) using nucleoprotein or matrix-specific primers with further H5 and H7 subtyping followed by sequencing of the HA cleavage site. There are relatively few data on the detection of AIV directly in organs or swabs collected from infected chickens (1,3,4,7). However, progress in this field is of great importance to further improvement of AI diagnosis.
The aim of the study was to apply RT-PCR for the detection of AIV in different tissues of experimentally infected chickens and compare the results to virus isolation method.

Materials and methods

Virus. AIV H7N1/AFR.STAR./983/79 strain was kindly provided by VLA Weybridge and used for chicken inoculation.
Experimental design. Four 4-week-old SPF chickens (Valo-Lohmann, Germany) kept in isolation were inoculated intraocularly and intranasally with 10^6 EID_{50} of the virus. Five days post inoculation (p.i.) tracheal and cloacal swabs as well as tissue samples from trachea, lung, liver, spleen, heart, brain, kidney, bursa of Fabricius, duodenum, caecal tonsils, rectum were collected. Supernatants of the organs (used for viral isolation as well as for RT-PCR) were prepared according to the Annex III of the Council Directive 92/40/EEC (2). Tracheal and cloacal swabs were suspended in PBS with antibiotics (1 ml/swab) and after 1 hour of incubation at room temperature and centrifugation, supernatants were harvested. All supernatants were pooled in batches of four. Additionally, the pooled supernatants of trachea, lungs, liver, spleen, kidneys, heart and brain (pooled sample N°1) and duodenum, caecal tonsils and rectum (pooled sample N°2) were also used as separate samples.
Virus isolation. Virus isolation (VI) was carried out on 9-11-day old embryonated SPF eggs inoculated into allantoic cavity according to the Annex III of the Council Directive 92/40/EEC (2). Two blind passages were performed.
RNA extraction and reverse transcription - polymerase chain reaction. RNA was extracted from supernatants using commercial test (Qiagen®) according to the manufacturer's instructions. Reverse transcription (RT) was performed for 50 min. at 42ºC in a total volume of 20 µl (5µl RNA, 0,1 µg of

hexamers, 200 µM of dNTP, 4 µl of reaction buffer (5x), 0,1 M DTT, 20U of ribonuclease inhibitor, 200U of reverse transcriptase). The sequences of primers to amplify the fragment of NP gene were described by Lee *et al.*(5). PCR was carried out in a reaction mixture (50 µl) containing 5 µl of cDNA, 5µl of PCR buffer 10x, 1 µl of dNTPs (25 mM each), 4 µl of $MgCl_2$ (25 mM), 1,5 Taq polymerase (Fermentas, Lithuania) and 2,5 µl of primers. The amplification conditions were: 94^0C for 3 min (initial denaturation), 35 cycles of 94^0C for 60 s (denaturation), 55^0C for 60 sec (annealing), 70^0C for 60 s (elongation) followed by 70^0C for 10 min (final elongation). The PCR products (expected size 330 bp) were separated on 1,5 % agarose gel.

Results and discussion

The results are shown in Tab.1. AIV was detected by RT-PCR in the cloacal swabs, caecal tonsils, rectum, bursa of Fabricius and pooled sample Nº2. Virus isolation yielded positive results in the cloacal swabs, trachea, caecal tonsils, bursa of Fabricius and pooled samples Nº1 and Nº2. We found 80% concordance between the results of VI and RT-PCR. These are preliminary results of the studies that were aimed to check the usefulness of the method. However, further collaborative inter-laboratories studies including optimization and validation of molecular techniques are needed. At the moment various laboratories use different methods of RNA isolation, primers targeting different genes (NP, M, HA), apply different RT and PCR conditions and PCR-product detecting systems (electrophoresis, PCR-ELISA) (1,3,7). All these stages are extremely important and influence the final effect, what was investigated by Starick & Werner (7) with special regard to RNA isolation methods, extension time and number of PCR cycles. However, an RT-PCR approach for the detection of AIV in tissue samples can be used alternatively to VI method. The reduction of time needed to obtain a final result to 24h is the greatest advantage that cannot be overestimated.

Table 1. Comparison of RT-PCR and virus isolation for the detection of AIV in tissues of experimentally infected chickens

	Cloacal swabs	Tracheal swabs	Pooled sample Nº1	Pooled sample Nº2	Trachea	Lungs	Duodenum	Caecal tonsils	Rectum	Liver	Spleen	Heart	Brain	Kidneys	Bursa of Fabricius
VI	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+
PCR	+	-	-	+	-	-	-	+	+	-	-	-	-	-	+

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Experimental infection of pigeons with HPAI H7N7 (The Netherlands 2003 virus)

Wendy Shell
Avian Virology
Veterinary Laboratories Agency

Veterinary Laboratories Agency

Background Information

- March 2003 – HPAI outbreak in The Netherlands
- Spread to Belgium and Germany
- Plans to hold pigeon show within the restricted area
- Demands from veterinary authorities to know risk

Previous Experiments 1

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Previous Experiments 2

- Panigraphy, B. et al., (1996) Susceptibility of pigeons to avian influenza. Avian Diseases 40, 600-604
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- Li, K.S. et al., (2004) Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 430, 209-213

MATERIALS AND METHODS

- **BIRDS**
- 20 pigeons – split into 2 groups
- 15 for infection
- 5 for controls/contacts
- **VIRUS**
- HPAI H7N7 derived from an outbreak of chickens in The Netherlands
- IVPI = 2.94

MATERIALS AND METHODS

- EXPERIMENTAL INFECTION
  - 15 pigeons were infected with 0.05ml (virus 10^7) intranasally
  - 3 contact birds added to the infected group 3 days post infection
  - The two remaining birds of the control/contact group remained as uninfected controls

Virus Isolation 1

- Re-isolation carried out on day 2, 4, 6, 8, 10, 13, 16 and 21
- Cloacal swabs placed in antibiotic broth for 1 hour
- 0.2ml of suspension inoculated into allantoic cavity of 9 to 10-day old embryonated fowls eggs
- Eggs candled daily, dead or chilled eggs tested for HA activity

Virus Isolation 2

- Two birds culled on day 6 and day 10
- Samples of intestine, heart, trachea, lung, liver, brain and kidney cut up and placed in antibiotic broth for one hour
- 0.2ml of suspension inoculated into allantoic cavity of 9 to 10-day old embryonated fowls eggs
- Eggs candled daily, dead or chilled eggs tested for HA activity

Re-isolation of virus in eggs

- Swabs
  - Passage 1 – no HA activity in any samples
  - Passage 2 – Low titres (2^2) of non specific HA in a few samples
- Tissues
  - Passage 1 – some HA activity seen in a some samples
  - Passage 2 – All samples negative for HA activity

Histological Examination

- Samples of intestine, heart, trachea, lung, liver, brain and kidney from culled birds fixed in formalin
- Examined for abnormalities by VLA Lasswade

Histological Results

- Mild acute to sub-acute focal hepatitis seen in the liver of 3 of the 4 birds
- Mild focal lymphocytic infiltrations seen in other tissues
- Conclusion – the lesions were considered to be non specific and commonly seen in 'normal' pigeons

Serological Tests

- Serum samples collected pre-infection, on culling and at the termination of the experiment
- Tested by HI test using 4HA units of antigen and doubling dilutions of serum

Results – All samples had HI titres of $<2^1$

Clinical Examination

- Pigeons examined daily for any clinical symptoms

Further Work

- Extraction and PCR of swab and tissue samples
- Passage 3 for samples showing HA activity on passage 2
- Comparison of results from histology of non contact/non infected birds with histology from culled infected birds
- A neutralisation assay will be performed on the serum samples

Conclusions 1

- Pigeons failed to show any clinical signs after infection with HPAI H7N7 virus
- There was no evidence of the pigeons excreting the virus – this to be confirmed by PCR
- There was no seroconversion of the virus

Conclusions 2

- Pigeons are unlikely to become infected with AI viruses
- Host range may be related to specific virus strains
- Pigeons could act as mechanical vectors of infective faecal material

Acknowledgements

- Defra – for funding of the project
- Guus Koch – for supplying clinical material used in this study
- Ruth Manvell for performing all the swabbing
- June Mynn for assisting with the swabbing and laboratory tests

Control of Low Pathogenic H7N2 Avian Influenza in Multiage Layers in Connecticut by Conventional and DIVA Vaccination Strategies

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[2]USDA, ARS, Southeast Poultry Research Laboratory, Athens, GA
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[5]CT Department of Agriculture, Hartford, CT

Objectives

- Background information
- Key elements of the control program
- DIVA, other test options
- Preliminary DIVA validation data
- Summary of results to date

USDA-APHIS

CT LPAI H7N2
Background

- Early March 2003 – LPAI H7N2 diagnosed in 3 of 4 table-egg layer farms owned by single company (approximately 3.88 million birds)
- Drop in egg production of about 35%
- H7N2 virus shown to be related to isolates found in the live-bird market system in northeastern US

USDA-APHIS

H7N2 Outbreaks Linked to Live-bird Markets

- 1996-98: PA (21 flocks, 2.5 M birds)
- 2001: CT (1 flock, 16,000 birds)
- 2001-2002: PA (7 flocks)
- 2002: VA, WV, NC (210 flocks, 4.7 M birds
- 2003: CT (4 flocks, 3.9 M layers)
- 2004: DE, MD (3 flocks, 200,000 + 210 K DC)

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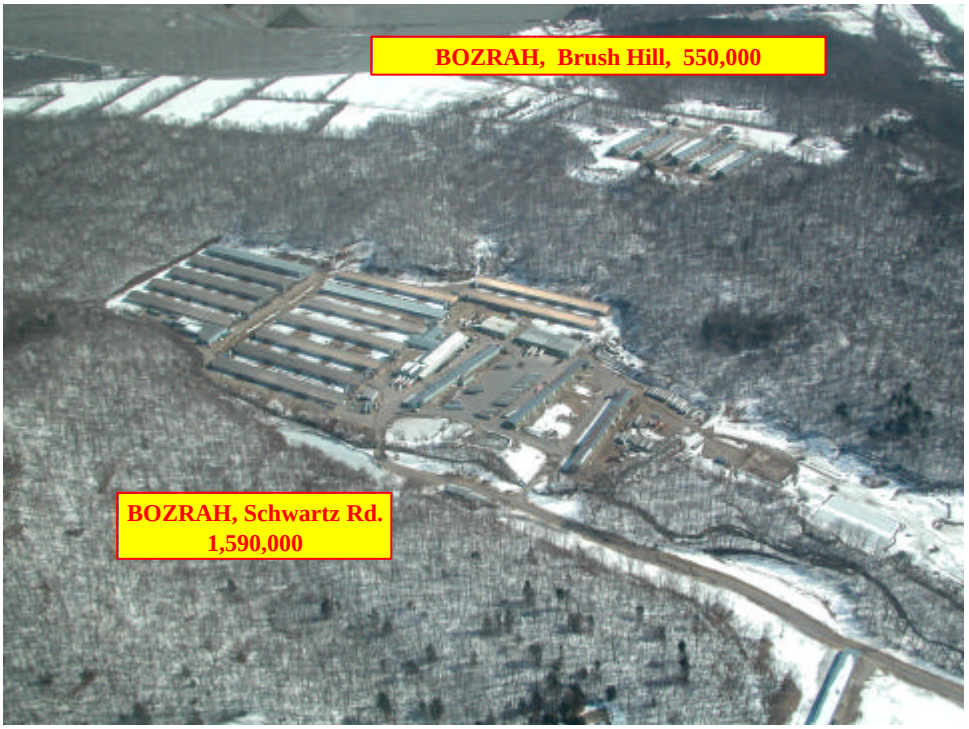
LEBANON, Mack Rd., 1,300,000


BOZRAH, Brush Hill, 550,000
BOZRAH, Schwartz Rd.
1,590,000

CT LPAI H7N2
Background (cont'd)

- Initial recommendation by USDA was to depopulate infected premises
  - ✓Based on previous experience with AIV in multi-age, continuous production flocks (PA)
  - ✓History of the LBM H7N2 AIV lineage
- March 15, 2003 – State vet requested permission from USDA to vaccinate – didn't have $16M to depopulate
- Series of discussions with experts from VS, academia, industry

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CT LPAI H7N2
Background (cont'd)

- Decision to develop pilot project
- Goal – vaccinate infected and replacement birds with H7 and heterologous NA subtype to utilize DIVA testing
  - ✓Ck/PA/96 H7N2 (3.2 million doses)
  - ✓Tk/UT/95 H7N3 (95% sequence homology)
  - ✓Initiated 4/16/04

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Key Elements of the Pilot Project

- Developed of Memorandum of Understanding between the State of CT and USDA
  - ✓Vaccination protocol
  - ✓Monitoring
  - ✓Exit guidelines
- Biosecurity review to prevent spread and/or reintroduction
- Disposal of birds
- Validation by CT Dept. of Ag

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Vaccination Protocol
Replacement Pullets and Layers

Non Infected

- 2 vaccinations 4 weeks apart
- Last vaccination at least 2 weeks prior to movement into lay house complex
- Serologic monitoring following vaccination

Infected

- Single vaccination
- Serologic monitoring following vaccination

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Pilot Project Guidelines
Spent Hens

- **3 weeks prior to depopulation**
  - ✓ One day/week – test daily mortality (up to 30 birds) by VI & RRT-PCR
- **Disposal**
  - ✓ Transport by covered trucks (prescribed routes)
    - ➢ Landfill
    - ➢ Rendering

USDA-APHIS

Pilot Project Guidelines
Manure

- Remain on premises until risk is determined by testing specimens from birds and/or manure (VI)
- 3 weeks prior to depopulation
  - ✓One day/week – test daily mortality (up to 30 birds) by VI & RRT-PCR

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Pilot Project Guidelines
House Cleaning and Disinfection

- Depopulate house
- Remove manure
- Blown, swept and dry cleaned
- Washed with high pressure washer
- Disinfected
- Environmental samples collected and tested (VI) by CT DOA

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Sentinel Birds
Pullet Houses

- 80 birds/house (leg banded)
- Tested by AGID, HI , RRT-PCR, VI
- After 2 weeks – 30 sentinels and 30 vaccinated birds routinely tested by HI
- Testing repeated after each vaccination
- When moved into laying house – randomly placed within house

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Monitoring
Laying Houses

Vaccinates and Infected

- One day/week – Tracheal swabs from daily mortality (min 10 birds) tested by Directigen, RRT-PCR and VI
- 20 sentinel birds monitored every 2 weeks by HI, RRT-PCR, and VI

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Monitoring *Neighboring Premises*

- 30 serum or yolk/house tested monthly by AGID
- Production drops – trigger diagnostic testing
- Mortality and production records monitored weekly

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Why use DIVA (Differentiating Infected fom Vaccinated Animals)?

- Increasing interest in using vaccine (LPAI)
- Increasing international acceptance to trade antibody-positive birds if antibody due to vaccination
- AGID and ELISA cannot identify vaccinated birds
- Use of heterologous neuraminidase vaccine provides opportunity to use DIVA to demonstrate that antibodies are due to vaccination

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Options for NA Antibody Testing Using DIVA

- **Baculovirus expressed N2**
  - ✓IFA
  - ✓ELISA
- **Neuraminidase inhibition test (Gold Standard)**
- Measure for NA antibodies to the circulating virus, e.g. N2
  - ✓Positive N2 indicates antibody to field virus (H7N2)
  - ✓Negative N2 indicates antibody to vaccine (H7N3)

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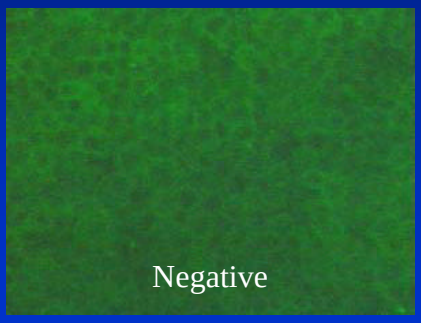
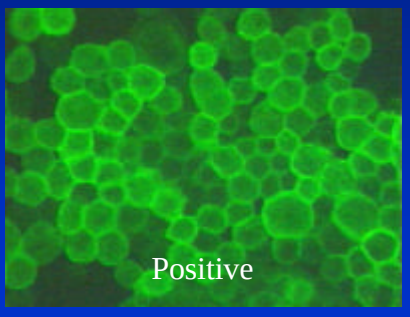
Baculovirus Expressed N2 Neuraminidase IFA Test

- Sucessfully used in Italy (H7N1)
- N2 baculovirus construct developed by Suarez *et al.*





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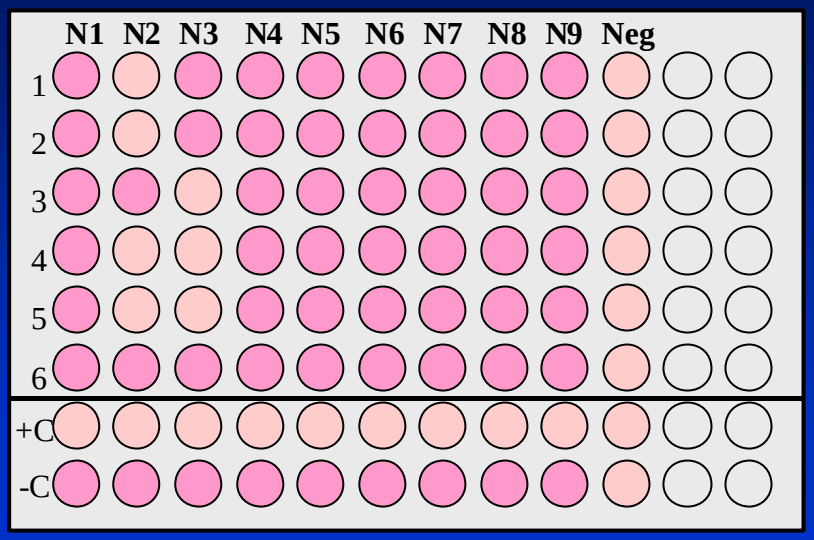
AIV Neuraminidase-Inhibition Test



DIVA Validation

- Collected 75 serums from various scenarios
  - ✓Not infected, no vaccination
  - ✓Infected, vaccinated with H7N2
  - ✓Infected, vaccinated with H7N3
  - ✓Not infected, vaccinated with H7N2
  - ✓Not infected, vaccinated with H7N3
- Tested by AGID, HI, NI, DIVA (IFA)

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DIVA Validation Data

	Non infected Vacc H7N2 (58 wk-old)	Non infected Vacc H7N3 (25 wk-old)	Infected Vacc H7N2 (46 wk-old)	Infected Vacc H7N3 (69 wk-old)
AGID	7/74 pos	73/74 pos	69/75 pos	75/75 pos
HI (H7)	53 /74 pos GMT=1:7	74/74 pos GMT=1:124	73/74 pos GMT=1:38	75/75 pos GMT=1:1154
NI (N2, 3)	69/74 pos N2 74/74 neg N3	66/66 neg N2 65/66 pos N3	74/74 pos N2 74/74 neg N3	72/75 pos N2 73/75 pos N3
DIVA (N2)	44/74 pos N2	73/73 neg N2	68/74 pos N2	75/75 pos N2

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DIVA vs. NI

	NI +	NI –
DIVA +	**184**	**3**
DIVA –	**32**	**97**

n = 316
DIVA (IFA 1:20)

Dx Sen = 85%
Dx Sp = 97%

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Results

- All serologic and virologic tests on sentinel birds have been negative
- All virologic tests on daily mortality testing of layers (VI and RRT-PCR) have been negative
- All DIVA and NI tests on H7N3-vaccinated birds have been negative for N2 antibodies

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Summary

- To date, the Pilot Project instituted to control LPAI in multi-age layer facility in CT using conventional vaccination and a DIVA strategy has been successful
  - ✓No serologic evidence in sentinels or DIVA vaccinated layers
  - ✓No virologic evidence of continued circulation of the H7N2 virus
- Last flock vaccinated September 2004

USDA-APHIS


Thank You
Acknowledgements:
Dr. Robert Brady
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Dr. Sandy Bushmich
CT vaccine working group

OUTBREAKS of H5 and H7 AVIAN INFLUENZA 1994-2004

Dennis Alexander
Community Reference Laboratory
Veterinary Laboratories Agency, UK

HIGHLY PATHOGENIC AVIAN INFLUENZA

PREVALENCE

- Has there been an increase in HPAI outbreaks in recent years [since 1994]?

Primary HPAI outbreaks in poultry since 1959

A/chicken/Scotland/59 (H5N1)
A/turkey/England/63 (H7N3)
A/turkey/Ontario/7732/66 (H5N9)
A/chicken/Victoria/76 (H7N7)
A/chicken/Germany/79 (H7N7)
A/turkey/England/199/79 (H7N7)
A/chicken/Pennsylvania/1370/83 (H5N2)
A/turkey/Ireland/1378/83 (H5N8)
A/chicken/Victoria/85 (H7N7)
A/turkey/England/50-92/91 (H5N1)
A/chicken/Victoria/1/92 (H7N3)
A/chicken/Queensland/667-6/94 (H7N3)
A/chicken/Mexico/8623-607/94 (H5N2)
A/chicken/Pakistan/447/94 (H7N3)
A/chicken/NSW/97 (H7N4)
A/chicken/Hong Kong/97 (H5N1)
A/chicken/Italy/330/97 (H5N2)
A/turkey/Italy/99 (H7N1)
A/chicken/Chile/2002 (H7N3)
A/chicken/Netherlands/2003 (H7N7)
A/chicken/SE Asia/2003 (H5N1)
A/chicken/Texas/2004 (H5N2)
A/chicken/Canada-BC/2004 (H7N3)
A/ostrich/S. Africa/2004 (H5N2)

Primary HPAI outbreaks in poultry since 1959

- 16/24 since 1985
- 13/24 since 1994
- 6/24 since 2000

Primary HPAI outbreaks in poultry since 1994

1994-1999	2000-2004
A/chicken/Queensland/667-6/94 (H7N3)	A/chicken/Chile/2002 (H7N3)
A/chicken/Mexico/8623-607/94 (H5N2)	A/turkey/Netherlands/2002 (H7N3)
A/chicken/Pakistan/447/94 (H7N3)	A/chicken/Netherlands/2003 (H7N7)
A/chicken/NSW/97 (H7N4)	A/chicken/SE Asia/2003 (H5N1)
A/chicken/Hong Kong/97 (H5N1)	A/chicken/Texas/2004 (H5N2)
A/chicken/Italy/330/97 (H5N2)	A/chicken/Canada-BC/2004 (H7N3)
A/turkey/Italy/99 (H7N1)	A/ostrich/S. Africa/2004 (H5N2)

Primary HPAI outbreaks in poultry in EU countries since 1959

- 6/11 1959-1993
- 3/13 1994-2004

DISTRIBUTION

- Has there been a change in geographical distribution of HPAI outbreaks in recent years [since 1994]?

Primary HPAI outbreaks since 1959

- 17/24 in chickens 6/24 in turkeys

By geographical area:

- 5/24 British Isles
- 5/24 Australia
- 2/24 Italy
- 4/24 North America
- 1/24Germany, Pakistan, Hong Kong [China], Mexico [Central America] Chile, The Netherlands [Belgium, Germany] SE Asia [9 countries], South Africa

Primary HPAI outbreaks since 1959

By geographical area before 1994:

- 5/11 British Isles
- 3/11 Australia
- 1/11 Germany, USA, Canada

By geographical area since 1994:

- 2/13 Italy & Australia
- 1/13 Pakistan, Hong Kong [China], Mexico [Central America] Chile, The Netherlands [Belgium, Germany], SE Asia [9 countries], USA, Canada, South Africa

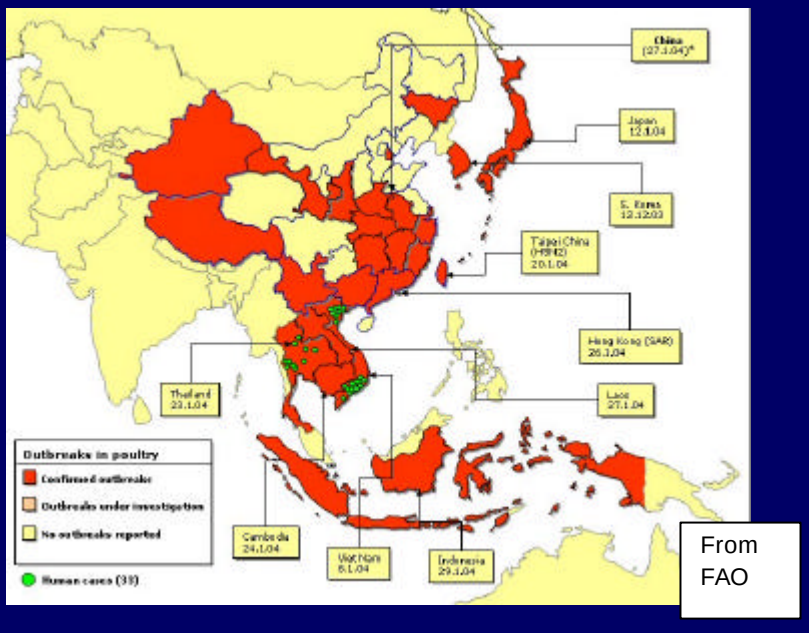
SPREAD

- Have HPAI outbreaks tended to be more widespread when they have occurred?

Primary HPAI outbreaks in poultry in EU countries since 1959

A/chicken/Scotland/59 (H5N1) - no spread?
A/turkey/England/63 (H7N3) - limited spread
A/chicken/Germany/79 (H7N7) - ?
A/turkey/England/199/79 (H7N7) - limited spread
A/turkey/Ireland/1378/83 (H5N8) - limited spread
A/turkey/England/50-92/91 (H5N1) - no spread
A/chicken/Italy/330/97 (H5N2) - limited spread
A/turkey/Italy/99 (H7N1) - widespread outbreaks
A/chicken/Netherlands/2003 (H7N7) – widespread outbreaks

UNPRECEDENTED? SPREAD IN EAST ASIA



From FAO

FAO estimations of number of birds involved in H5N1 HPAI in SE Asia

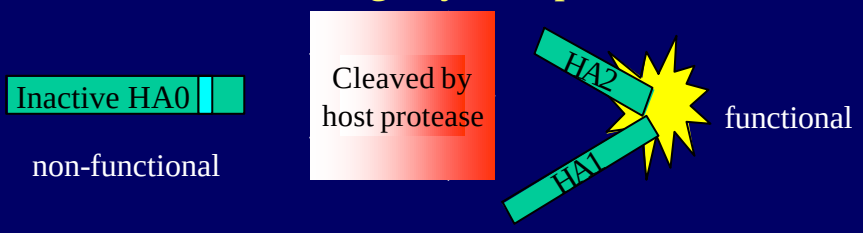
Country	No birds infected/culled
S. Korea	~250,000 [>5,000,000 culled]
Viet Nam	36,000,000
Japan	~100,000
Thailand	36,000,000
Cambodia	~7,500
Lao PDR	~3,000
Indonesia	15,000,000
China	~8,000,000

HPAI VIRUSES

- Q - Is there anything different about recent HPAI viruses?
- A - No but….

Influenza haemagglutinin protein

- Mediates fusion between virus and host cell membranes
- Synthesised as an inactive precursor HA0
- Activated via cleavage by host proteases



Amino acids at the H cleavage site of H7 influenza A viruses

H7 viruses of low pathogenicity

- European & Asian -PEIPKGR<>GLF-
- American -PENPKGR<>GLF-

H7 Viruses of high pathogenicity - examples

- ty/England/63 -PETPKRRRR<>GLF-
- ck/Australia/76 -PEIPKKREKR<>GLF-
- ty/England/199/79 -PEIPKKREKR<>GLF-
- ck/Pakistan/447/95 -PETPKRKRKR<>GLF-
- ck/Australia/CR2/95 -PEIPRKRKR<>GLF-

Virulence of avian influenza viruses

- The presence of multiple basic amino acids at the HA0 cleavage site means the viruses are able to be cleaved by a ubiquitous protease & spread systemically in all tissues
- Without additional basic amino acids at the cleavage site the viruses are restricted to replication in the respiratory and intestinal tracts

EMERGENCE OF HPAI

- Current theories are that mutation from LPAI to HPAI takes place AFTER introduction of the LPAI virus to poultry from wild birds.

Garcia et al (1996), Perdue et al (1998)

HPAI in CHILE 2002

A/chicken/Chile/2002 (H7N3)

IVPI 2.8-2.98

Also LPAI H7N3 isolate

HA0 cleavage site amino acid sequences of H7N3 HPAI Chile isolates

PEKPKTCSPLSRCRETR*GLF (4372)
PEKPKTCSPLSRCRKTR*GLF (4957)
10 amino acid insert
30 nucleotides from nucleoprotein gene

HPAI in Canada [British Columbia] 2004

- Virus H7N3 IVPI 2.8
- Slaughtered all poultry in Fraser Valley = ~17 million birds
- HA0 cleavage sequence:

PENPKQAYRKRMTRGLF

21 nucleotide insert from the matrix gene

HPAI IN USA 2004

- February 2004 HPAI H5N2 confirmed in Gonzales County, Texas
- Broiler flock 6,608 birds
- Does not appear to be the same virus as the H5N2 in Mexico
- Confirmed as HPAI on molecular characterisation PQRKKRGLF since the virus did not kill inoculated chickens IVPI 0.0
- Extensive surveillance indicated no further outbreaks

LOW PATHOGENICITY AVIAN INFLUENZA

H5 subtype low pathogenicity avian influenza viruses in the EU 1994-2004

1994	ratites	Netherlands	H5N9
1996	ostriches	Netherlands Q	H5N2
1996	ostriches	Denmark Q	H5N2
1999	chickens	Belgium	H5N2
2003	ducks	Denmark	H5N7
2003	broilers	France	H5N2

H7 subtype avian influenza viruses of low pathogenicity in the EU 1994-2003

1998	turkeys [28] chickens [1]	Ireland	H7N7
1998	turkeys [2] chickens [1]	N. Ireland	H7N7
1999-01	poultry [199]	Italy	H7N1
2002	turkeys [2?]	Netherlands	H7N3
2002-2003	poultry [288]	Italy	H7N3

CONTROL

CONTROL OF HPAI

- Control of HPAI has been almost exclusively by strict biosecurity, movement restrictions and eradication by stamping out.
- Exceptions are Mexico and Pakistan and some Asian countries

Examples of control of recent H5 and H7 LPAI infections 1

- 1996/7 Pennsylvania LPAI H7N2
  - controlled by biosecurity, depopulation
- 1998 Ireland LPAI H7N7
  - controlled by biosecurity, voluntary slaughter
- 1999 Belgium LPAI H5N2
  - controlled by slaughter
- 1999 Italy LPAI H7N1
  - control by biosecurity - HPAI emerged

Examples of control of recent H5 and H7 LPAI infections 2

- 2000/1 Italy LPAI H7N1 re-emerged
  - controlled by DIVA vaccination + stamping out
- 2001 Germany LPAI H7N7
  - Stamped out
- 2002 Virginia LPAI H7N2
  - Stamped out

Examples of control of recent H5 and H7 LPAI infections 3

- 2002 Connecticut LPAI H7N2
  - Vaccination [and stamping out]
- 2003 Italy LPAI H7N3
  - controlled by DIVA vaccination + stamping out
- 2003 Denmark LPAI H5N7 in com. ducks
  - Stamped out

Control of recent H5 and H7 LPAI infections

Of 10 listed above

- 6 stamped out
- 3 voluntary slaughter/depopulation
- 1 biosecurity alone (Italy 1999 – HPAI emerged)

Control measures in SE Asia

Korea	Stamping out
Japan	Stamping out
Thailand	Stamping out
Lao PDR	Stamping out
Cambodia	Stamping out
China	Stamping out & vaccination
Viet Nam	Modified stamping out
Indonesia	Modified stamping out & vaccination

VACCINATION

Desired results of vaccination against AI

- freedom from disease
- no effect on production or other serious expense
- no trade embargoes
- eradication

Avian influenza vaccination

Current vaccines result in:

- Protection against clinical signs
- Reduction in virus excretion
- Increase in virus dose needed to infect bird

BUT……

AI vaccination

- AI virus may infect and replicate in vaccinated birds without clinical signs
- As a corollary HPAI as defined by OIE may still be confirmed in such birds
- Infection with HPAI virus without clinical signs may lead to spread and an endemic situation

Recent successful use of vaccination against H5 or H7 AI

Country	year	virus	Other control measure
Italy	2000/1	H7 LPAI	Stamping out
Italy	2002/3	H7 LPAI	Stamping out
USA (CT)	2003	H7 LPAI	Stamping out

Recent unsuccessful use of vaccination against H5 or H7 AI

Country	year	virus	Other control measure
Mexico	1994-	H5 LPAI/HPAI	none

LPAI H5 virus continues to circulate

Pakistan	1995-	H7 HPAI	none

HPAI H7 virus continues to circulate

Survey for AI in poultry and wild birds in the EU - update

Ian Brown
Community Reference Laboratory
Veterinary Laboratories Agency, UK

Programme objectives

- To investigate the prevalence of infections with influenza A viruses of H5 and H7 subtypes in different species of poultry
- To contribute to a cost–benefit study in relation to eradication of all H5 and H7 subtypes from poultry envisaged by the change in definition of avian influenza
- To take the preliminary steps towards the connection and integration of human and veterinary networks for influenza surveillance

Implementation/progress 04

- Guidelines for programmes reviewed and issued (subject to annual review)
- Member states programme prepared according to guidelines, submit to commission and approved
- Financial support for 50% of cost incurred of approved programme
- Results to be submitted to CRL by **15.3.05** for collation

General structure of programme

- National Reference Laboratories
  - liaise with veterinary authorities for poultry survey
  - laboratory tests using standard antigens from CRL
  - collate results and submit to CRL
  - wild bird surveillance if required (optional)
    - submit any viruses isolated from wild birds to CRL

General structure of programme

- Community reference laboratory
  - technical support, guidelines, protocols
  - supply of standardised reagents to NRL's
  - verification of laboratory results if required
  - functions as NRL
  - standard tests for H5/H7 viruses
    - characterisation of submitted viruses
  - data collation from NRL's and production of final report
  - integration of veterinary and human networks for influenza surveillance

Hosts

- Major hosts in member states
  - include ducks, geese, fattening turkeys, chicken and turkey breeders, layers, farmed game birds, ratites, quail
- Outdoor production focus
- Host susceptibility to influenza A virus

Sampling

Statistical requirements

- Identify at least 5% prevalence with 95% (99% -turkeys) confidence interval
- 95% probability of i/d positive birds assuming 30% seroprevalence in flock
- 10 birds per farm

Samples and testing

- Blood samples for serological examination
- Collected at abattoir/on farm
- HI test with H5 and H7 antigens according to Directive 92/40/EC
  - Initial screening using validated assays permitted if in approved programme
- Two stage HI testing
  - To eliminate NA cross reactive antibody
- International standard for interpretation of positives

Antigenic analyses of H5 viruses by HI

	Antisera (chicken)			
Virus	ty/On/66	os/Dk/96	ck/Be/99	dk/Fr/02
ty/On/66	**1024**	8	128	nd
ty/En/91	64	256	64	nd
os/Dk/96	16	**128**	16	nd
ck/It/98	32	32	16	64
ck/Be/99	128	128	**256**	nd
dk/Fr/02	16	16	16	**256**
dk/Dk/03	32	64	32	256
ck/Th/04	16	64	4	16

Evaluation of serology for commercial anseriformes

- 'Ring trial'
  - Panel of 58 sera derived from 11 holdings (range 1-18)
  - 4 laboratories
- Serology using HI +/- ELISA
- Standard procedures
- HI titres > or equal to 16 considered positive

Results of ring trial for serology on anseriformes

- Good reproducibility within laboratory when:
  - HI titres > or equal to 64
  - Same antigens/greater number of samples per holding
- ELISA (2 labs) 43/48 positive influenza A
- Good qualitative consistency between laboratories
- Holding status
  - 3 negative for H5/H7
  - 4 positive for H7
  - 3 positive for H5
  - 1 positive for H5 and H7

Recommendations/Conclusions

- Serology by HI for anseriformes in 2004 programme
- 40-50 samples should be tested per holding to compensate for apparent reduced sensitivity of HI
- Same testing protocol as for samples from other poultry hosts. All positive samples should be subject to rigorous retesting before positive holdings are declared
- Derogation to do VI on cloacal samples should be retained as an alternative approach
- Archiving sera from ducks/geese
  - Please forward to CRL

Survey guidelines for wild birds

- Use of ornithological groups/societies
- Diversity of species
  - waterfowl, shorebirds & other free-living birds
- Seasonal focus, migratory routes
- Virus detection using faecal material
  - test sample pools from same host species
- All AI virus isolates should be submitted to CRL

COUNTRY REPORTS ON AVIAN INFLUENZA FOR 2003 BASED ON RESPONSES TO THE QUESTIONNAIRE

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INTRODUCTION

Continuing the format adopted at the 7th Meeting the information for this report was taken from answers supplied by National laboratories to the following questionnaire:

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AVIAN INFLUENZA

1. How many samples from which species of bird/type of poultry have been processed that would have resulted in the isolation of avian influenza viruses in eggs and in cell culture?

Example response:

broilers	*200 cloacal swabs*	*in eggs*
	60 tissue samples	*in eggs*
turkeys	*100 cloacal swabs*	*in eggs*
	140 tissue samples	*in eggs*
	140 tissue samples	*in cell cultures*

2. State the number of influenza viruses isolated, their subtype, and the type of bird from which they were isolated.

Example response:

meat turkeys	*3 x H6N2*
	2 x H9N2
waterfowl	*2 x H4N6, 1 x H5N2*

3. For all influenza viruses isolated state type of poultry or species of bird and IVPI. For H5 and H7 isolates give amino acid sequence at the HA0 cleavage site and conclusion.

Example response:

Bird	***subtype***	***IVPI***	***HA0 cleavage site***	***conclusion***
Turkeys	*H9N2*	*0.00*	*nd*	*LPAI*
feral duck	*H5N2*	*0.00*	*PQRETR*GLF*	*LPAI*

4. Was any active surveillance for avian influenza carried out? If so give details of birds sampled, number of samples and results.

***

RESULTS

A total of 33 questionnaires was sent to different laboratories in 30 countries. Responses were received for 23/25 EU countries: Austria, Italy, Portugal, Spain, Greece, Ireland, UK, Denmark, Finland, France, The Netherlands, Sweden, Germany, Belgium, Cyprus, Estonia, Latvia, Slovenia, Hungary, Luxembourg, Poland, Lithuania, Czech Republic, and from 5/5 non-EU countries: Norway, Bulgaria, Turkey, Romania and Switzerland. The samples tested and the results for avian influenza are summarised in the following pages.

VIRUS ISOLATION REPORTS BY COUNTRY

AUSTRIA

Type of bird	Sample	Method	Number
chickens	tissue samples	in eggs	852
	cloacal swabs	in eggs	98
turkeys	tissue samples	in eggs	2
geese	cloacal swabs	in eggs	280
ducks	cloacal swabs	in eggs	10
wild birds	cloacal swabs	in eggs	333

Influenza viruses isolated
None.

BELGIUM

Samples tested

Type of bird	Sample	Method	Number
poultry	tissue samples	in eggs	1320
chickens & turkeys	tissue samples	in cell culture	1320
ducks & geese	cloacal swabs	in eggs	43
pigeons	tissue samples	in eggs	45
	tissue samples	in cell culture	45
quarantine birds	cloacal swabs	in eggs	400

Influenza viruses isolated
Layers 4 x H7N7; broilers 2 x H7N7; breeders 1 x H7N7; meat turkeys 1 x H7N7

Examples of virus characterisation

Bird	***subtype***	***IVPI***	***HA0 cleavage site***	***conclusion***
Meat turkeys	H7N7	2.95	PEIPKTRRRR*GLF	HPAI
Chickens	H7N7	2.95	PEIPKTRRRR*GLF	HPAI

BULGARIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Method	Number
broilers	tissue samples	in eggs	22
pigeons	tissue samples	in eggs	2
pheasants	tissue samples	in eggs	3

Influenza viruses isolated
None.

Serological monitoring for avian influenza antibodies

Type of bird	Number of samples	Method	Result
chickens	1186	ELISA	negative
waterfowl	139	ELISA	negative
turkeys	50	ELISA	negative
pigeons	55	ELISA	negative
pheasants	90	ELISA	negative

CYPRUS

Samples tested by inoculation into eggs:

Type of bird	Sample	Method	Number
broilers	tissue samples	in eggs	5
pigeons	tissue samples	in eggs	4
flamingos	tissue samples	in eggs	14
cage birds	tissue samples	in eggs	3
geese	tissue samples	in eggs	2
sea gulls	tissue samples	in eggs	3
ducks	tissue samples	in eggs	2
pea cock	tissue samples	in eggs	1

Influenza viruses isolated
None.

Serological monitoring for avian influenza antibodies

Type of bird	Number of samples	Method	Result
chickens	148	AGID	negative
ostriches	30	AGID	negative

CZECH REPUBLIC

Samples tested by inoculation into eggs

Type of bird	Sample	Method	Number
layers	tissue samples	in eggs	1
partridges	tissue samples	in eggs	2
swans	tissue samples	in eggs	2
goose	tissue samples	in eggs	1
zoo birds	tissue samples	in eggs	1

Influenza viruses isolated
None

Serological monitoring for avian influenza antibodies

Type of bird	No. flocks	No. of samples	Method	Result
broilers	29	298	ELISA	negative
layers	5	57	ELISA	negative
turkeys	6	60	ELISA	negative
ducks	2	20	AGID HI H5/7	negative
others	14	37	AGID HI H5/7	negative

DENMARK

Samples tested by inoculation into eggs

Type of bird	Sample	Number
domestic fowl	tissues	413
caged birds	tissues	774
ducks and geese	cloacal swabs tissues	77 175
game birds	tissues	112
turkeys & ostriches	tissues	56
pigeons	tissues	21
wild birds	faeces	2895

Influenza viruses isolated.
Domestic ducks: 1 x H5N7.
Wild birds: 1 x H1N1, 2 x H3N2, 4 x H3N8, 5 x H4N6. 1 x H6N5, 1 x H6N8, 1 x H10N7.

Virus characterisation

Bird	***subtype***	***IVPI***	***HA0 cleavage site***	***conclusion***
farmed mallard	H5N7	0	PQKETR*GLF	LPAI

ESTONIA

Samples tested
None

Serological monitoring for avian influenza antibodies

Type of bird	No. flocks	No. of samples	Result
various	13	2743	all negative

FINLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	faeces	8
	tissues	1
turkeys	faeces	12
	tissues	2
ducks	cloacal swabs	37
pheasants	faeces	61
	tissues	22
cage birds	faeces	5
	tissues	4
pigeons	faeces	1
	tissues	1
water & shore birds	faeces	212
	tissues	8
other wild birds	faeces	32
	tissues	3

Influenza viruses isolated
None.

FRANCE

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
domestic fowl	cloacal swabs	48
	tissues	10
turkeys	cloacal swabs	46
	tissues	1
ducks	cloacal swabs	24
	tissues	4

guinea fowl	tissues	3
goose	tissues	1
ornamental birds	cloacal swabs tissues	1 31
wild birds	tissues	5

Influenza viruses isolated

Bird	***subtype***	***IVPI***	***HA0 cleavage site***	***conclusion***
turkeys(4)	H6N2	0.0	not done	LPAI
turkeys	H9N9	0.0	not done	LPAI
ducks	H6N2	0.0	not done	LPAI
broilers	H5N2	0.0	PQRETR	LPAI

GERMANY

Samples tested

Type of bird	**Sample**	**Method**	**Number**
chickens	tissues cloacal swabs	eggs eggs	609 111
turkeys	tissues	eggs	96
ducks	tissues cloacal swabs	eggs eggs	44 3240
geese	tissues cloacal swabs	eggs eggs	28 3640
backyard poultry, ornamental chickens	tissues cloacal swabs	eggs eggs	221 196
pigeons	tissues	eggs cell cultures	736
psittacine birds	tissues	eggs	433
pet birds, zoo birds	tissues cloacal swabs	eggs eggs	85 2229
wild birds	tissues cloacal swabs	eggs eggs	85 1750

Influenza viruses isolated

Chickens 1 x H7N7
Ducks 1 x H6N1
Geese 1 x H6N2
Wild birds 3 x H2, 1 x H3, 3 x H4, 3 x H5, 2 x H7, 5 x H10, 2 x H13

Characteristics of isolated influenza viruses

Bird	Subtype	IVPI	HA_0 cleavage site	Conclusion
chickens	H7N7	2. 93	^{337}KRRRR*GLF	**HPAIV**
ducks	H6N1	0.00	nd	LPAIV
geese	H6N2	0.00	nd	LPAIV
feral ducks (A. platyrh.)	H2N3	nd	nd	LPAIV
feral ducks (A. platyrh.)	H2	nd	nd	LPAIV
feral ducks (A. platyrh.)	H2	nd	nd	LPAIV
feral ducks (A. platyrh.)	H3N8	nd	nd	LPAIV
feral ducks (A. platyrh.)	H4N6	nd	nd	LPAIV
feral ducks (A. platyrh.)	H4N6	nd	nd	LPAIV
feral ducks (A. platyrh.)	H4N6	nd	nd	LPAIV
feral ducks (A. platyrh.)	H5N2	0.00		LPAIV
feral ducks (A. platyrh.)	H5N3/N2	0.00	^{339}RETR*GLF	LPAIV
feral ducks (A. crecca)	H5N2	0.00		LPAIV
feral ducks (A. platyrh.)	H7N1	0.00	^{337}KGR*GLF	LPAIV
feral ducks (A. platyrh.)	H7N3/N7	0.00		LPAIV
feral ducks (A. platyrh.)	H10	nd	nd	LPAIV
feral ducks (A. platyrh.)	H10	nd	nd	LPAIV
feral ducks (A. platyrh.)	H10	nd	nd	LPAIV
feral ducks (A. platyrh.)	H10N7	nd	nd	LPAIV
feral ducks (A. platyrh.)	H10N7	nd	nd	LPAIV
moorhen	H10N4	nd	nd	LPAIV
gull	H13N6	nd	nd	LPAIV
jackdaw	H13N6	nd	nd	LPAIV

Serological monitoring for avian influenza antibodies

10 samples per flock at slaughter tested by IDEXX ELISA. Positive samples were subtyped by HI test

Type of bird	No. of samples	Positive	Subtypes
chickens	4590	4 flocks	2 x H3, 2 x H6
meat turkeys	1700	0	-
ducks	1430	1 flock	1 x H6
geese	2050	5 flocks	1 x H3, 1 x H4, 2 x H6, 1 x H12
other poultry & zoo birds	542	0	-

GREECE

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues cloacal swabs	55 112
broiler breeders	cloacal swabs	46
layers	tissues cloacal swabs	28 142
meat turkeys	tissues cloacal swabs	32 65

Influenza viruses isolated
None

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
broilers	1930 694	AGID ELISA	negative negative
layers, breeders and turkeys	634	AGID	negative

HUNGARY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	54
hens	tissues	4
turkeys	tissues	2
ducks	tissues	2
pheasant	tissues	2
pigeons	tissues	8

Influenza viruses isolated
None

IRELAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broiler	tissues	5
layers	tissues	6
'chickens'	tissues	18
turkeys	tissues	11
pheasants	tissues	6
geese	tissues	14
ducks	tissues	6
pigeon	tissues	30
exotic	tissues	10
peacock	tissues	1
wild birds	cloacal swabs	127
other birds	tissues	40

Influenza viruses isolated
wild bird 1 x H10N5

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
broiler	50 726	AGID HI [H5/7]	negative negative
broiler breeder	19,192 490	AGID HI [H5/7]	negative negative
layer	1,138 530	AGID HI [H5/7]	negative negative
pedigree layer	120 0	AGID HI [H5/7]	negative negative
chicken	483 108	AGID HI [H5/7]	negative negative
commercial turkey	1082 532	AGID HI [H5/7]	negative negative
turkey breeder	1230 70	AGID HI [H5/7]	negative negative
goose	75 85	AGID HI [H5/7]	negative negative
exotic	2 0	AGID HI [H5/7]	negative negative
pheasant	1 0	AGID HI [H5/7]	negative negative
pigeon	7 0	AGID HI [H5/7]	negative negative
duck	0 15	AGID HI [H5/7]	negative negative
unstated	1 0	AGID HI [H5/7]	negative negative

ITALY

Samples tested in eggs:

Type of bird	Sample	Number
broiler breeders	tracheal swabs	4
broilers	cloacal swabs tracheal swabs	9 6
layers	tracheal swabs cloacal swabs tissues	3 3 2
rural chickens	tissues	6
turkey breeders	tracheal swabs	4
meat turkeys	tissues tracheal swabs cloacal swabs	5 96 127
pheasants	cloacal swabs	60
guinea fowl	tracheal swabs cloacal swabs	1 1
ostriches	pools of cloacal swabs	64
domestic ducks	cloacal swabs	102
domestic geese	cloacal swabs	10
wild ducks	cloacal swabs for RTPCR	478 25 +ve in eggs
pigeons	tissues	2
grey partridge	cloacal swabs	10
peacock	tissues	2
pet birds in Q	cloacal swabs	10
quail	cloacal swabs	3

Influenza viruses isolated

Meat turkeys 43 x H7N3 (36 from IZSVE + 7 isolated in other laboratories)
Broilers 5 x H7N3 (1 from IZSVE, 4 others)
Broiler breeders 3 x H7N3 (2 from IZSVE, 1 other)
Layers 1 x H7N3 (from IZSVE)
Guinea fowl 2 x H7N3 (1 from IZSVE, 1 other)
Domestic ducks 1 x H7N3 (isolated in other laboratory)
Pet birds in quarantine 3 x H3N8 (isolated in other laboratories)
Domestic geese 1 x H1N1 (isolated in other laboratory)
Mallard 1 x H1N1
Pin tail [Anas acuta] 1 x H10N4

Characterisation of viruses isolated in Italy

Birds	*Subtype*	*IVPI*	*HA0 cleavage site*	*Conclusion*
Meat turkey	H7N3	nd	PEIPKGR*GLF	LPAI
Boiler	H7N3	nd	PEIPKGR*GLF	LPAI
Broiler breeders	H7N3	nd	PEIPKGR*GLF	LPAI
Layers	H7N3	nd	PEIPKGR*GLF	LPAI
Domestic duck	H7N3	nd	PEIPKGR*GLF	LPAI
Guinea fowl	H7N3	nd	PEIPKGR*GLF	LPAI
Pet birds	H3N8	nd	not done	LPAI
Domestic geese	H1N1	nd	not done	LPAI
Mallard (*Anas platyrhynchos*)	H1N1	nd	not done	LPAI
Pintail (*Anas acuta*)	H10N4	nd	not done	LPAI

LATVIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	20
layers	tissues	125

Influenza viruses isolated
none

Serological monitoring for avian influenza antibodies

Type of poultry	Method	Number of flock tested	Number of sera examined	Number of flock positive	Number of sera positive
Layer	ELISA, IDEXX	8	290	-	-
Broilers		2	87	-	-
Layer	HAAR (H5 & H7), Russian	7	75	-	-
Broilers		1	3	-	-
Zoo birds		1	5	-	-
Layer	AGID VLA	8	305	-	-
Broilers		1	40	-	-
Zoo birds		1	4	-	-

LITHUANIA

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
poultry [24 farms]	1155	ELISA	negative

LUXEMBOURG [tests done by Belgium**]**

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
poultry?	tissues	13

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
poultry	118	HI	negative

THE NETHERLANDS

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
'poultry'	tracheal swabs tissues faeces	84 343 5
chickens	tracheal swabs tissues faeces	620 1567 30
turkeys	tracheal swabs tissues	164 167
ducks	tissues cloacal swabs	40 10
geese	tissues cloacal swabs	3 11
ostriches	tissues	2
pigeons	tissues faeces	6 3
miscellaneous	tracheal swabs tissues faeces	12 16 1
unknown	tracheal swabs tissues faeces	30 106 1
caged birds quarantine	cloacal swabs faeces	512 221

Influenza viruses isolated

poultry	110 x H7N7
chickens	1006 x H7N7
turkeys	205 x H7N7; 5 x H7N3
ducks	5 x H7N7
ostriches	1 x H7N7; 1 x H2N3
misc.	10 x H7N7

Characterisation of viruses isolated

Bird	subtype	IVPI	HA0 cleavage site [a]	conclusion
chickens	H7N7	2.94	PEIPKRRRR *GLF	HPAI
turkeys	H7N7	N.D.	PEIPKRRKR *GLF[b]	HPAI
chickens	H7N7	N.D.	PEIPKRRKR *GLF[c]	HPAI
turkeys	H7N3	2,4[d]	PEIPKGR*GLF	LPAI
ducks	H7N7	nd	PEIPKRRRR *GLF	HPAI
ostrich	H7N7	nd	PEIPKRRRR *GLF	HPAI
ostrich	H2N3	0	VPQIESR*GLF?	LPAI

[a] *The haemagglutinin gene of 71 of a total of 244 HPAI H7 virus isolates was sequenced partially. Only H7N7 isolates with mutations in the cleavage site motif are listed. Sequence was obtained from RNA directly isolated from organ suspensions.*

[b] *RNA isolated from brain suspension. Brain was used in this case because ND was suspected Virus isolated form trachea had the motif PEIPKRRRR *GLF.*

[c] *RNA isolated from trachea suspension.*

[d] *Virus re-isolated from IVPI chickens had motif PEIPKGSRVRR*GLF.*

NORWAY

Samples tested by inoculation into eggs and detection by RTPCR:

Type of bird	Sample	Number
wild duck	cloacal swabs	3
wild pigeon	cloacal swabs	119
domestic pigeon	cloacal swabs	11
wild geese	cloacal swabs/faeces	200

Influenza viruses isolated

1 x H3N8 from a wild duck [HA0 cleavage site PEKQTR*GL]

Serological monitoring for avian influenza antibodies

Type of bird	No. of flocks	No. of samples	Method	Result
chicken (imports)	7	210	HI (H5/H7)	negative
chicken	100	1000	HI (H5/H7)	negative
turkey (imports)	3	90	HI (H5/H7)	negative
turkey	3	30	HI (H5/H7)	negative
wild pigeons		110	HI (H5/H7)	negative

POLAND

Samples tested by inoculation into eggs

Type of birds	Sample	Method	Number
Commercial layers	tissues	eggs	5
broilers	tissues	eggs	5
turkeys	tissues	eggs	5
pheasants	cloacal and tracheal swabs	eggs	68
peacocks	cloacal and tracheal swabs	eggs	4
geese	faeces/cloacal swabs	eggs	1740
ducks	faeces/cloacal swabs	eggs	390
wild birds	faeces/cloacal swabs	eggs	363

Influenza viruses isolated
none

Influenza serology – routine diagnosis

Type of birds	Number of flocks tested		Number of sera examined		Number of flocks positive		Number of sera positive	
	H5	H7	H5	H7	H5	H7	H5	H7
broiler breeders	18	30*	915	1078	0	0	0	0
broilers	3	3	69	69	0	0	0	0
pheasants	2	2	17	17	0	0	0	0
peacocks	2	2	7	7	0	0	0	0

* some of the flocks were imported from Holland and Germany

PORTUGAL

Samples tested by inoculation into eggs or cell culture:

Type of bird	Sample	Method	Number
broilers	faeces	eggs	2
	tissues	eggs	5
	tissues	cells	1
chickens	tissues	eggs	9
partridges	faeces	eggs	27
	tissues	eggs	29
exotic birds	tissues	eggs	2
	tissues	cells	1
exotic birds	faeces	eggs	59
in quarantine	tissues	eggs	22
pigeons	tissues	eggs	6
ostriches	faeces	eggs	2
pheasants	faeces	eggs	6
feral ducks	cloacal swabs	eggs/cells	167
	faeces		2
shorebirds	faeces	eggs	11
quail	faeces	eggs	1
wild birds	faeces	eggs	22

Influenza viruses isolated
None

Serological monitoring for avian influenza antibodies

Type of bird	No. of samples	Method	Result
layers	507	ELISA/HI	negative
broiler breeders	512	ELISA/HI	negative
broilers	113	ELISA/HI	negative
meat turkeys	1125	ELISA/HI	negative
quails	215	HI	negative
ducks		HI	negative
ostriches		HI	negative

ROMANIA

Samples tested by inoculation into eggs

Type of bird	Sample	Number
broilers	cloacal swabs	1251
broilers	tissues	10
pigeons	tissues	1

Influenza viruses isolated
None

Serological monitoring for avian influenza antibodies

Type of bird	No. of samples	Method	Result
chickens	53339	AGID	negative

SLOVENIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	5
broiler breeders	tissues	2
meat turkeys	tissues	10
pigeons	tissues	2
ostrich	tissues	1

Influenza viruses isolated
None

SPAIN

Samples tested by inoculation into eggs:

Type of bird	***Sample***	***Number***	***Result***
American kestrel	cloacal swabs	6	negative
barn owl	cloacal swabs	15	negative
black crowned night heron	cloacal swabs	1	negative
booted eagle	cloacal swabs	3	negative
Buteo buteo	cloacal swabs	8	negative
chicken	cloacal swabs	10	negative
cattle egret	cloacal swabs	1	negative
canary	cloacal swabs	637	negative
common coot	cloacal swabs	1	negative
cock	cloacal swabs	4	negative
crow	cloacal swabs	1	negative

duck	cloacal swabs	38	negative
eagle	cloacal swabs	2	negative
European nightjar	cloacal swabs	1	negative
flamingo	cloacal swabs	5	negative
gannet	cloacal swabs	5	negative
glossy ibis	cloacal swabs	1	negative
goshawk	cloacal swabs	1	negative
grey heron	cloacal swabs	6	negative
owl	cloacal swabs	11	negative
ostrich	cloacal swabs	23	negative
partridge	cloacal swabs	1	negative
pigeon	tissue samples cloacal swabs	9 6	negative
cage birds including quarantine	cloacal swabs	5810	negative
purple gallinula	cloacal swabs	1	negative
red kite	cloacal swabs	2	negative
roseate spoonbill	cloacal swabs	1	negative
seagull	cloacal swabs	10	negative
Spanish imperial eagle	cloacal swabs	5	negative
stork	cloacal swabs	14	negative
tawny owl	cloacal swabs	5	negative
toucan	cloacal swabs	7	negative
turkey	cloacal swabs	1	negative
turtle dove	cloacal swabs	60	negative
vulture	cloacal swabs	6	negative

Influenza viruses isolated
None.

SWEDEN

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broiler breeders	tissues	15
layers		
broilers	tissues	1
backyard poultry	cloacal swabs	10
pigeons	tissues	28
wild birds	tissues	10
zoo birds	tissues	7

Influenza viruses isolated
None

Serological monitoring for avian influenza antibodies

Type of poultry	***Flocks tested***	***Sera examined***
imported broiler breeders in isolation	11	220
imported layer breeders in isolation	3	60
layers	60	600
imported turkey breeders in isolation	5	100
meat turkeys	24	240
free range hens	2	20
ostriches	2	10
wild birds		2

All results were negative.

SWITZERLAND

Samples tested by inoculation into eggs:

Type of bird	**Sample**	**Number**
laying hens	tissues	4
broilers	tissues	38
pigeons	tissues	7
pet birds	tissues	3
pheasant	tissues	9
duck	tissues	11
quail	tissues	13

Influenza viruses isolated
None.

Serological monitoring for avian influenza antibodies [using the IDEXX-ELISA for AI].

Type of poultry	**Flocks tested**	**Sera examined**	**Flocks positive**	**Sera positive**
turkeys	4	40	-	-

TURKEY

Negative report

UNITED KINGDOM - GREAT BRITAIN

Samples tested

Type of bird	Sample	Method	Number
chickens	tissues	eggs	595
	tissues	cell cultures	210
	cloacal swabs	eggs	21
	cloacal swabs	cell cultures	4
turkeys	tissues	eggs	111
	tissues	cell cultures	100
	cloacal swabs	eggs	12
	cloacal swabs	cell cultures	8
pet birds	tissues	eggs	431
	tissues	cell cultures	95
	cloacal swabs	eggs	25
	cloacal swabs	cell cultures	4
game birds	tissues	eggs	161
	tissues	cell cultures	35
	cloacal swabs	eggs	30
	cloacal swabs	cell cultures	31
pigeons	tissues	eggs	193
	tissues	cell cultures	167
	cloacal swabs	eggs	32
	cloacal swabs	cell cultures	17
waterfowl	tissues	eggs	87
	tissues	cell cultures	77
	cloacal swabs	eggs	8
	cloacal swabs	cell cultures	4
raptors	tissues	eggs	39
	tissues	cell cultures	11
	cloacal swabs	eggs	32
	cloacal swabs	cell cultures	4
other birds	tissues	eggs	50
	tissues	cell cultures	27

In addition 850 swabs from commercial waterfowl [170 pools] for survey, plus 413 individual cloacal swabs for wild bird survey.

Influenza viruses isolated

1 x H6N2 from ducks, also 1 x H3N8 from commercial waterfowl and 1 x H9N9 from wild birds in surveys.

UNITED KINGDOM – NORTHERN IRELAND

Samples tested by inoculation into eggs

Type of bird	Sample	Number
chickens	tissues	76
	cloacal swabs	18
turkeys	tissues	6
	cloacal swabs	30
	tracheal swabs	30
pigeons	tissues	8

Influenza viruses isolated
None.

DISCUSSION

The questionnaire for 2003 continued the trend of more countries responding seen over recent years. The responses for the last 4 years compared to the number of countries invited to complete the questionnaire have been:
2000 19/29; 2001 22/29; 2002 25/30; 2003 28/30.

The diagnostic and surveillance work for avian influenza done in Europe in 2003 was very much affected by the HPAI H7N7 outbreaks in The Netherlands, Belgium and Germany and the LPAI H7N3 outbreaks in Italy. In marked contrast to these 19 countries [20 including N. Ireland] failed to detect any avian influenza viruses in the samples tested.

The overall isolation attempts for avian influenza are summarised in Table 1 for egg inoculations and Table 2 for cell culture inoculations. The overall total of 45,866 was up on the total of 35,374 for 2002 and is more than 5 times the total of 8,498 in 2001.
A total of 48 LPAI influenza viruses of subtypes other than H5 or H7 was isolated from six countries (Table 3). Thirty two of these isolates were obtained from wild birds and a further three from caged quarantine birds. Isolated from commercial birds were restricted to ducks geese and ostriches, likely to be reared on open range and turkeys. There were no isolates of these viruses from chickens.

The H5 and H7 subtype influenza viruses isolated during 2003 are summarized in Table 4. The large numbers isolated in Italy and The Netherlands reflect the outbreaks of LPAI and HPAI seen respectively in those countries and for the latter there was spread into Belgium and Germany. Only Germany report H5 and H7 infections of wild ducks, although H5 infections were reported in broilers in France and farmed mallards in Denmark.

Table 1 Summary of virus isolation attempts in eggs by countries responding to the questionnaire

Type of bird	Number countries reporting attempts	Total all samples*
chickens	26	10,972
turkeys	14	1,096
ducks & geese	15	9,812
game birds	12	767
ostriches	4	103
pigeons	15	1,228
cage, zoo, pet, quarantine etc	14	9,937
wild birds	13	8,649
others	5	336
	TOTALS	**42,930**

***tissues/tracheal swabs/cloacal swabs/faeces**

Table 2 Summary of virus isolation attempts in cell cultures by countries responding to the questionnaire

Type of bird	Number countries reporting attempts	Total all samples
chickens	3	1,535
turkeys	1	108
ducks & geese	1	4
game birds	1	35
pigeons	3	964
cage, zoo, pet, quarantine etc	2	96
wild birds	1	179
others	1	15
	TOTALS	**2,936**

* tissues/tracheal swabs/cloacal swabs/faeces

Table 3 Summary of non-H5 or H7 LPAI viruses isolated by countries responding to the questionnaire

Type of bird	Subtype	No. of isolates	No. Countries
turkeys	H6N2	4	1
	H9N9	1	1
com. ducks	H6N1	1	1
	H6N2	3	3
	H3N8	1	1
com. geese	H1N1	1	1
	H6N2	1	1
ostriches	H2N3	1	1
cage Q birds	H3N8	3	1
wild ducks	H1N1	2	2
	H2N?	2	1
	H2N3	1	1
	H3N2	2	1
	H3N8	6	3
	H4N6	6	2
	H6N5	1	1
	H6N8	1	1
	H9N9	1	1
	H10N?	3	1
	H10N4	1	1
	H10N7	2	2
other wild birds	H10N4	1	1
	H10N5	1	1
	H13N6	2	2

Table 4 Summary of H5 or H7 subtype AI viruses isolated.

Country	Subtype	Bird	Number	Virulence
Belgium	H7N7	meat turkeys, chickens	8	HPAI
Denmark	H5N7	farmed mallard	1	LPAI
France	H5N2	broilers	1	LPAI
Germany	H5N?	wild ducks	1	LPAI
	H5N2	wild ducks	2	LPAI
	H7N?	wild ducks	1	LPAI
	H7N1	wild ducks	1	LPAI
	H7N7	chickens	1	HPAI
Italy	H7N3	meat turkeys, chickens, domestic duck, Guinea fowl	54	LPAI
The Netherlands	H7N3	turkeys	5	LPAI/HPAI
	H7N7	poultry, chickens, turkeys, ducks, ostriches, miscellaneous	1337	HPAI

Annual Meeting of the National Laboratories for Newcastle disease

TECHNICAL REPORT FOR THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2003

I. LEGAL FUNCTIONS AND DUTIES

The functions and duties are specified in Annex V of Council Directive 92/66/EEC (Official Journal of the European Communities No L 260 of 5.9.1992).

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2002

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decision 95/233/EC (Official Journal of the European Communities No L 156, p. 76) as amended by Decision 96/619/EC (OJ No L 276, p. 18). This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:

   a) Determining the intracerebral pathogenicity index (ICPI)

   b) Determining basic amino acids composition adjacent to the cleavage site of the F0 protein in the virus

   c) Antigenic grouping of viruses

   d) Limited phylogenetic analysis to assist in epidemiological investigations

***Work Plan**: The number of viruses received will be dependent on the outbreaks occurring and those viruses submitted, as a guide the numbers received since 1988 are shown in Table 1.*

Table 1. Number of viruses submitted to the CRL each year since 1988

1988	1989	1990	1991	1992	1993	1994	1995
401	188	113	154	199	294	385	605
1996	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**
284	227	285	357	704	316	333	464

The identification of all viruses received will be confirmed. All ND viruses will be subjected to antigenic grouping using monoclonal antibodies. ICPI tests will be done if not already assessed in the National Laboratories at the request of the NL or the Commission. Nucleotide sequencing and phylogenetic studies will be carried out on representative viruses.

***% Resources:** 70 %*

WORK DONE: The 464 viruses submitted in 2003 were characterised as shown in Table 2.

Table 2: Identification of viruses submitted to the CRL in 2003

Virus identification	Number
Influenza A viruses	**155**
Paramyxoviruses	**166**
APMV-1 [NDV]	135
APMV-2	10
APMV-3	8
APMV-4	6
APMV-6	2
APMV-8	2
APMV-9	3
not yet typed	112
virus not viable	31

In addition to identification, 24 intracerebral pathogenicity index tests were done on ND viruses at the request of the submitting country.

All APMV-1 viruses were also assessed using a panel of monoclonal antibodies to determine antigenic and epizootiological relationships. For 24 representative APMV-1 viruses the nucleotide sequence of an area of the fusion protein gene from the signal sequence through the cleavage site was obtained for *in vitro* assessment of virulence and use in phylogenetic studies.

Estimated actual % resources: 71%

2. Maintain and distribute virus repository and reagents necessary for virus characterisation.

Work Plan*: Maintenance of existing repository will continue. All viruses submitted to the CRL will be added to the repository after characterisation. Most viruses will be maintained in a frozen state, but selected, representative viruses will be freeze dried. Reagents such as polyclonal chicken sera, monoclonal antibodies and control antigens will be maintained at levels that previous demands have indicated to be necessary.*

% Resources: *12 %*

WORK DONE: The 135 ND viruses received were added to the repository. Reagent stocks were maintained, at least at previous levels [Table 3] and during the year the following were supplied:

For the year 2003 63 x 1.0ml ampoules of Newcastle disease (ND) antigen and 80 x 0.5ml ampoules of ND antiserum were supplied. In addition 1 x 1.0ml of

APMV-2, 3 x 1.0ml of APMV-3, and 2 x 1.0ml of APMV-6, antigen, and 1 x 0.5ml of APMV-2, 14 x 0.5ml of APMV-3, and 2 x 0.5ml of APMV-6 antiserum were distributed.
Certain ND virus specific monoclonal antibodies were also supplied to different laboratories: mAb 85 11 x 0.5 ml, mAb 161 40 x 0.5 ml and mAb 7D4 22 x 0.5 ml.

Estimated actual % resources**: **12%

Table 3. Stocks of polyclonal chicken sera and virus antigens for HI tests held at the Community Reference Laboratory.

Type	Serum		Antigen	
	Quantity[a]	HI titre[b]	Quantity[a]	HA titre[b]
SPF	100	<1		
NDV	100	8	50	8
APMV-3	150	8	100	8

[a] Number of freeze-dried ampoules containing 0.5 ml of serum or antigen at the indicated titre.
[b] HI and HA titres are expressed as $\log_2$. The SPF serum had an HI titre of <1 to each antigen.

3. Prepare and distribute antisera, antigens and reagents for the inter-laboratory comparison tests.

***Work Plan**: Antisera and antigens to be used in the comparison tests will be prepared, freeze-dried and dispatched to the National Laboratories in time for results to be reported at the next annual meeting.*

***% Resources:** 6 %*

WORK DONE: Antigens and antisera were prepared and dispatched to EU National Laboratories and those of accession countries [total 31 laboratories]

Estimated actual % resources**: **5%

4. Analysis of results submitted by National Laboratories for the inter-laboratory comparison tests.

***Work Plan**: As in previous years, results submitted by the National laboratories will be analysed and presented at the next annual meeting.*

***% Resources:** 2 %*

WORK DONE: Results were received, analysed and an oral presentation made at the Annual Meeting in 2003. A written report will appear in the proceedings.

Estimated actual % resources**: **2%

5. Conduct work to evaluate reported problem areas in diagnosis.

***Work Plan**: Staff of the CRL will be available for consultation by National Laboratories, problem sera and other reagents will be received from National Laboratories for testing and evaluation.*

% Resources: *1 %*

WORK DONE: Staff of the CRL were consulted on an ad hoc basis.

Estimated actual % resources**:* ***1%

6. Supporting by means of information and technical advice National Newcastle Disease Laboratories and the European Commission during epidemics.

Work Plan: Staff of the CRL will be available for consultation and forward all relevant information to the National Laboratories or the Commission, as appropriate.

% Resources: *2 %*

WORK DONE: Staff of the CRL were consulted on numerous occasions by other National Laboratories, representatives of member states and the Commission.

Estimated actual % resources**:* ***2%

7. Prepare programme and working documents for the Annual Meeting of National Newcastle Disease Laboratories.

***Work Plan**: The organisation of the Annual Meeting in collaboration with the Commission's representative will be done as in previous years.*

% Resources: *2 %*

WORK DONE: In collaboration with the Commission's representatives the Annual Meeting was organised and held in Brussels in December 2003.

Estimated actual % resources**:* ***2%

8. Collecting and editing of material for a report covering the annual meeting of National Newcastle Disease Laboratories.

***Work Plan**: Receive and collate submissions edit and produce report of 2002 proceedings before 2003 Annual meeting. Receive and collate submissions of 2003 meeting.*

***% Resources**: 3 %*

WORK DONE: Proceedings of the 2002 meeting were produced.

Estimated actual % resources**: **3%

9. Preparation and publications of articles and reports associated with above work.

***Work Plan**: Results obtained relating to the work of the CRL will be published in the proceedings of the Annual Meeting or, where appropriate and with the permission of the Commission, submitted to international journals as scientific publications.*

***% Resources:** 2 %*

WORK DONE: The following publications appeared in 2003 relating to the work of CRL for ND

1. ALEXANDER, D.J. & MANVELL, R.J. (2003). CRL Technical Report for ND 2001. Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Padova, 2002 pp 74-78.
2. ALEXANDER, D.J. & MANVELL, R.J. (2003). Country Reports on ND based on questionnaires Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Padova, 2002 pp 79-87.
3. ALEXANDER, D.J. & MANVELL, R.J. (2003). Interlaboratory comparative tests. Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Padova, 2002 pp 94-99.
4. ALDOUS, E.W. & ALEXANDER, D.J. A molecular epidemiological investigation of isolates of the variant APMV-1 virus (PPMV-1) responsible for the 1978-2002 panzootic in pigeons. Abstracts of the XII International Conference on Negative Strand RNA Viruses. Pisa Italy June 14-19th 2003 p197.
5. ALEXANDER, D.J. (2003) Newcastle disease, Other Avian Paramyxoviruses and Pneumovirus infections: Introduction. In Diseases of Poultry. Y.M. Saif [ed in chief] Iowa State University Press USA pp 63-64.
6. ALEXANDER, D.J. (2003) Newcastle disease, Other Avian Paramyxoviruses and Pneumovirus infections: Newcastle disease. In Diseases of Poultry. Y.M. Saif [ed in chief] Iowa State University Press USA pp 64-87.

7. ALEXANDER, D.J. (2003) Newcastle disease, Other Avian Paramyxoviruses and Pneumovirus infections: Avian paramyxoviruses 2-9. In Diseases of Poultry. Y.M. Saif [ed in chief] Iowa State University Press USA pp88-92
8. ALDOUS, E.W., MYNN, J.K. BANKS, J. & ALEXANDER, D.J. (2003). A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. Avian Pathology, 32, 239-357.
9. COLLINS, M.S., GOVEY, S.J. & ALEXANDER, D.J. (2003) Rapid *in vitro* assessment of the virulence of Newcastle disease viruses using the ligase chain reaction. Archives of Virology 148, 1851-1862.

Estimated actual % resources**: **2%

COUNTRY REPORTS ON NEWCASTLE DISEASE AND OTHER APMV INFECTIONS FOR 2003 BASED ON RESPONSES TO THE QUESTIONNAIRE

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INTRODUCTION

Continuing the format adopted at the 7th Meeting the information for this report was taken from answers supplied by National laboratories to the following questionnaire:

***

NEWCASTLE DISEASE

1. How many samples from which species of bird/type of poultry have been processed that would have resulted in the isolation of paramyxoviruses in eggs and in cell culture?

Example response:

broilers	*200 cloacal swabs*	*in eggs*
	60 tissue samples	*in eggs*
pigeons	*100 cloacal swabs*	*in eggs*
	140 tissue samples	*in eggs*
	140 tissue samples	*in cell cultures*

2. State the number of paramyxoviruses isolated, their serotype, and the type of bird from which they were isolated.

Example response:

meat turkeys	*3 x APMV-1*
	2 x APMV-3
pigeons	*20 x APMV-1 [PPMV-1]*

3. For APMV-1 viruses state type of poultry or species of bird, ICPI, amino acid sequence at F0 cleavage site, mAb group if known and conclusion.

Example response:

Bird	***ICPI***	***amino acids***	***mAb group***	***conclusion***
broiler	*0.2*	*^{112}GRQGRL^{117}*	*E*	*vaccine*
turkeys	*1.82*	*^{112}RRQRRF^{117}*	*C1*	*Newcastle disease*
pigeon	*0.9*	*^{112}RRQKRF^{117}*	*P*	*PPMV-1*

4. Countries with a non-vaccinating status for ND only. Provide information on serological monitoring:-

Example response:

Type of poultry	***Number of flocks tested***	***Number of sera examined***	***Number of flocks positive***	***Number of sera positive***

RESULTS

A total of 33 questionnaires was sent to different laboratories in 30 countries. Responses were received for 23/25 EU countries: Austria, Belgium, Czech Republic, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, The Netherlands, Poland, Portugal, Slovenia, Spain, Sweden, UK, and from 5/5 non-EU countries: Bulgaria, Norway, Switzerland, Romania and Turkey. The responses for number of samples processed for ND [APMV-1] are identical to those for avian influenza virus isolations [see above] the results in terms of avian paramyxovirus isolates are summarised in the following pages.

***

VIRUS ISOLATIONS REPORTS BY COUNTRY

AUSTRIA

APMV isolates

laying hens [free-range] 1 x APMV-1
pigeons 3 x APMV-1

Characterisation of APMV-1 isolates

Bird	***ICPI***	***Amino acids***	***mAb group***	**Conclusion**
broiler	0.2	nd	nd	vaccine
pigeons	1.02	nd	nd	PPMV-1
pigeons	1.05	nd	nd	PPMV-1
pigeons	1.12	nd	nd	PPMV-1

BELGIUM

APMV isolates

pigeons 5 x APMV-1 [PPMV-1]
quarantine birds 1x APMV other than 1 or 3
layers 10 x lentogenic APMV-1 (8 x La Sota & 2 x Ulster vaccines)
meat turkeys 1 x lentogenic APMV-1 (La Sota vaccine)

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	Conclusion
pigeon	n.d.	^{112}RRQKRF^{117}	P	PPMV-1
poultry	n.d.			lentogenic (vaccine) APMV-1

BULGARIA
No isolates

CYPRUS
No isolates

CZECH REPUBLIC

APMV isolates
pigeons 1 x APMV-1

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
pigeon	0.45	nd	nd	PPMV1?

DENMARK

APMV isolates
ducks 3 x APMV-1
pheasants 1 x APMV-1
wild birds 1x APMV-1
caged birds 3 x APMV-2 1 x APMV-3 (exotic bird type)

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*[1]	*Conclusion*
pheasant	0.2	^{112}GKQGRL^{117}	C2 or G/Q	lp APMV1
Cygnus bewickii	ND	^{112}GKQGRL^{117}	C2 or G/Q	lp APMV1
mallards	1.16/0.0	^{112}GKQGRL^{117}	C2 or G/Q	confusion
mallard	ND	^{112}ERQERL^{117}	H	lp APMV1
duck	ND	^{112}GKQGRL^{117}	C2 or G/Q	lp APMV1

[1]Deduced from sequences of the F gene (cleavage site region)

ESTONIA
No isolates.

FINLAND

APMV isolates
pheasant 1 x APMV-1

Characterisation of APMV-1 isolates

Bird	***ICPI***	***Amino acids***	***mAb group***	***Conclusion***
pheasant	0.5	^{112}GKQGRL^{117}	nd	lentogenic APMV1

FRANCE

APMV isolates

meat turkeys	1 x APMV1
mule ducks	1 x APMV1; 1 APMV4
pigeons (ornamental)	1 x APMV1 [PPMV1]
wild pigeons	1 x APMV1 [PPMV1]
other ornamental birds	1 x APMV2

Characterisation of APMV-1 isolates

Bird	***ICPI***	***Amino acids***	***mAb group***	**Conclusion**
turkeys	0.0	GKQGRL	≠ La Sota	avirulent
ducks	0.0	GKQGRL	≠ La Sota	avirulent
pigeons(2)	1.2	RRQKRF	P	PPMV1

GERMANY

Characterisation of APMV-1 isolates

Bird	*No.*	ICPI	Amino acids	*mAb group*	*Conclusion*
chickens	5	not done	^{112}GRQGR*L^{117}	E	lentogenic/vaccine
ornamental chicken	1	0.88	^{112}RRQKR*F^{117}	P	PPMV-1
turkeys	2	not done	^{112}GRQGR*L^{117}	E	lentogenic/Vaccine
ducks	1	not done	^{112}GRQGR*L^{117}	E	lentogenic/Vaccine
	1	not done	^{112}GKQGR*L^{117}	?	lentogenic
pigeons	36	1.36 [35 x not done]	^{112}RRQKR*F^{117}	P	PPMV-1
	1	not done			lentogenic
psittacines	1	not done	not done	P	PPMV-1
	1	not done	not done	E	lentogenic/vaccine
pet birds	1	not done	not done	P	PPMV-1

Other APMVs

Bird	*Number*	*Type*
chicken	1	APMV-2
ostrich	1	APMV-3
psittacine	1	APMV-3

GREECE
No isolates

IRELAND

APMV isolates
pigeons 5 x APMV-1

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	*conclusion*
pigeon	1.05	NA	P	PPMV-1
pigeon	0.625	NA	P	PPMV-1
pigeon	0.8	NA	P	PPMV-1
pigeon	0.7	NA	P	PPMV-1
pigeon	0.78	NA	P	PPMV-1

ITALY

APMV isolates

Broiler	3 x APMV-1 (from IZSVE)
Meat turkey	1 x APMV-1 (from others)
Pigeon	11 x APPMV-1 (4 from IZSVE + 7 from others)
Collared dove	9 x PPMV-1 (4 from IZSVE + 5 from others)
Domestic duck	10 x APMV-1 (2 from IZSVE + 8 from others)
Rural chicken	7 x APMV1 (2 from IZSVE + 5 from others)
Pintail (*Anas acuta*)	1 x APMV-9 (from IZSVE)

Characterisation of APMV-1 isolates

Bird	**ICPI**	***Amino acid***	***mAb group***	***Conclusion***
broilers	0.1	GKQGR*L	ni*	1 x lentogenic
	0.4-0.6	GRQGR*L	ni	2 x lentogenic
domestic duck	0.1-0.2	GKQGR*L-ERQER*L	ni	7 x lentogenic
	0.5-0.68	GKQGR*L	ni	3 x lentogenic
rural chickens	0.1-0.2	GKQGR*L-GRQGR*L	ni	3 x lentogenic
	0.4-0.65	GRQGR*L	ni	2 x lentogenic
	1.6-1.8	RRQKR*F	ni	2 x NDV
pigeons	0.75 -1.8	KRQKR*F-RRQKR*F	P	11 x PPMV-1
collared doves	0.65 -1.3	RRQKR*F	P	9 x PPMV-1
meat turkeys	0.1	GKQGR*L	ni	1 x lentogenic

*ni = not identifiable

LUXEMBOURG

No isolates

LITHUANIA

No isolates

THE NETHERLANDS

APMV isolates

poultry (broilers)	2 x lentogenic APMV-1
turkey+ 4 chickens (BY?)	1 x lentogenic APMV-1
miscellaneous	1 x lentogenic APMV-1
exotic birds (Q)	11 x from 1 or more pools APMV-2
	1 x virulent APMV-1

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
exotic birds (Q)	1.65	^{112}RRQKRF^{117}	nd	virulent NDV
broilers	nd	^{112}GKQGRL^{117}	nd	lentogenic[a]

[a] The partial sequence is compatible with that of the Ulster strain and the isolate is thus most likely re-isolated vaccine virus.

NORWAY

APMV isolates
pigeons 5 x APMV-1

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
pigeon	0.4	^{112}RRQKRF^{117}	nd	PPMV-1

POLAND
No isolates

PORTUGAL

APMV isolates

pigeon 1 x APMV-1
feral ducks 4 x APMV-4

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	**Conclusion**
pigeon	nd	RRQKRF	P	PPMV-1

ROMANIA
No isolates.

SLOVENIA
No isolates

SPAIN

APMV isolates
wild turtle dove 1 x APMV-1

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	Conclusion
pigeon	0.22	nd	P	PPMV-1

SWEDEN

APMV isolates

backyard turkey	1 x APMV-1
parrot in zoo	1 x APMV-1
pigeons	8 x APMV-1

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
backyard turkey	1.25	RRQKRF	P	PPMV-1(NDV)
parrot in zoo	0.14	RRQKRF	P	PPMV-1
pigeons (3)	0.6-0.7	RRQKRF	P	PPMV-1
pigeons (5)	n.d.	n.d	P	PPMV-1

SWITZERLAND
No isolates.

TURKEY
No isolates.

UNITED KINGDOM [GREAT BRITAIN]

APMV isolates

pigeons/doves	29 x APMV-1
caged quarantine birds	3 x APMV-1
	8 x APMV-2
	7 x APMV-3

Characterisation of APMV-1 isolates

Bird	*ICPI*	*mAb group*	*Phylogenetic group*	*Conclusion*
cage birds Q	1.8 /1.78/1.8		5b	3 x NDV
pigeons	N.D.	29 x P		29 x PPMV-1

UNITED KINGDOM [NORTHERN IRELAND]
No isolates

Thirteen of the 28 countries participating reported no isolation of avian paramyxoviruses [9 EU and all 4 non-EU]. The other 15 laboratories reported a total of 226 avian paramyxoviruses. One hundred and eight-five of these were APMV-1 viruses (Table 1). Fifty-five of these APMV-1 viruses were of low virulence representing the isolation of live vaccine viruses or naturally occurring avirulent viruses. There were only two virulent APMV-1 viruses reported and these were obtained from rural chickens in Italy. However, isolates of PPMV-1 viruses that fell within the definition of virulent NDV were obtained from turkeys in Sweden and ornamental chickens in Germany. A further 120 isolates obtained from pigeons [108], doves [10] and cage birds [2] were identified as APMV-1 viruses responsible for the ongoing panzootic in pigeons [PPMV-1]. In all, 12 different countries reported PPMV-1 isolations and this emphasises the continued widespread presence of this virus in Europe

Table 1 Summary of APMV virus isolations reported

Type of APMV	***Bird***	***No. countries***	***No. isolates***
PPMV-1	pigeons	11	108
	collared doves	1	9
	turtle doves	1	1
	cage birds	3	2
	turkey	1	1
	ornamental chicken	1	1
virulent APMV-1	chickens	1	2
	psittacines	2	4
low virulence APMV-1	poultry	8	44
	wild birds	2	9
	cage birds	2	2
APMV-2	caged birds	4	22
	chickens	1	1
	ornamental bird	1	1
APMV-3	caged birds	3	9
	ostrich	1	1
APMV-4	wild waterfowl	1	4
	domestic mule ducks	1	1
APMV-6	wild waterfowl	1	1
APMV-9	wild waterfowl	1	1

SEROLOGY FOR APMV-1

Six countries with non-vaccinating policies reported surveillance for APMV-1 antibodies in unvaccinated birds using haemagglutination inhibition tests and their results are listed below:

DENMARK

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
fowl	299	17,330	3	13
ducks and geese	5	139	3	57
game birds	2	101	0	0
turkeys	1	49	0	0
pigeons	1	52	0	0
ostriches	7	53	2	3
other birds	27	66	2	2

ESTONIA

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
various	14	3171	0*	0

*193 sera were positive by ELISA, but negative by HI test.

FINLAND

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
broilers	69	3948	1	58
turkeys	9	526	0	0
layers	23	1210	0	0
geese	1	60	0	0

NORWAY

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
fowl	122	6679	-	4
turkeys	10	361	0	0
dom. geese	4	143	0	0
dom. ducks	6	304	0	0
pigeons	1	131	1	21

SWITZERLAND

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
laying hen	3	34	-	-
pet bird	-	13	-	-
pigeon*	1	10	1	10
peacock	1	10	-	-
ratites	1	2	-	-

*vaccination is allowed in pigeons

SWEDEN

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
imported broiler breeders in isolation	11	1060		
broiler breeders	92	5480		
imported layer breeders in isolation	7	700		
layer breeders	15	1067		
pigeons	1	18	1	9
imported turkey breeders in isolation	5	500		
turkey breeders	10	420		
backyard poultry	11	266	2	17
ostriches	3	14		
zoo birds		25		13

The occasional detection of positive flocks or individuals in these countries, with the absence of clinical signs or the isolation of virulent viruses probably represents introduction of viruses of low virulence from infected feral birds.

CONCLUSION

As in 2002 it can be concluded from the results reported in the returned questionnaires that there was an extremely low prevalence of ND [virulent APMV-1 infections] in European poultry in 2003. However, the continued presence of ND in the racing and feral pigeon/dove populations in Europe [an epizootic that now spans 22 years] remains a serious cause for concern and a continuing threat for domestic poultry and wild life.

AVIAN INFLUENZA AND NEWCASTLE DISEASE IN THE EUROPEAN UNION: LEGISLATIVE ASPECTS

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1. DISEASE NOTIFICATION AND SITUATION IN THE EU

1.1. AVIAN INFLUENZA

Table 1: Outbreaks reported by Member States by the ADNS (Animal disease notification system 2000-2004:

COUNTRY	2000	2001	2002	2003	2004
Austria	0	0	0	0	0
Belgium	0	0	0	**8**	0
Denmark	0	0	0	0	0
Finland	0	0	0	0	0
France	0	0	0	0	0
Germany	0	0	0	**1**	0
Greece	0	0	0	0	0
Ireland	0	0	0	0	0
Italy	**351**	0	0	0	0
Luxembourg	0	0	0	0	0
Netherlands	0	0	0	**241**	0
Portugal	0	0	0	0	0
Spain	0	0	0	0	0
Sweden	0	0	0	0	0
United Kingdom	0	0	0	0	0
TOTAL	**351**	**0**	**0**	**250**	**0**

1.2. NEWCASTLE DISEASE

Table 2: Outbreaks reported by Member States by the ADNS (animal disease notification system 2000-2004:

COUNTRY	2000	2001	2002	2003	2004
Austria	0	0	0	**2***	0
Belgium	0	0	0	0	0
Denmark	0	0	**135**	0	0
Finland	0	0	0	0	**1**
France	0	0	0	0	0
Germany	0	0	0	0	0
Greece	0	0	0	0	0
Ireland	0	0	0	0	0
Italy	**256**	**1**	0	**1**	0
Luxembourg	0	0	0	0	0
Netherlands	0	0	0	0	0
Portugal	0	0	0	0	0
Spain	0	0	0	0	0
Sweden	0	**1**	0	**1***	**2**
United Kingdom	0	0	0	0	0
TOTAL	**257**	**2**	**135**	**4**	**3**

* PPMV-1 infection in pigeons

1.3. DISEASE SITUATION IN INDIVIDUAL MEMBER STATES AND LEGISLATION IN THIS RESPECT

1.3.1. Avian Influenza

No outbreaks of highly pathogenic avian influenza (HPAI) as currently defined in EU legislation (Directive 92/40/EEC) have been reported during the period since the last annual meeting in 2003.

1.3.1.1. Low pathogenicity avian influenza (LPAI) and vaccination against AI in Italy

Vaccination against subtype AI subtype H7H3 has been carried out in a defined area of Northern Italy since the end of 2002. A DIVA vaccination strategy using a heterologous subtype H7N1 was employed accompanied by a stringent surveillance programme of vaccinated and unvaccinated flocks. Since September 2003 no further circulation of subtype H7N3 had been detected. However at the time of the meeting end September 2004 a re-emergence of subtype H7N3 occurred in turkey flocks in the area under restrictions (see contribution by G. Cattoli), which led to the continuation of the vaccination campaign.

The ongoing intensive surveillance activities showed also evidence of introduction of other LPAI subtypes respectively H1N1, H1N2, H3N8, H5N3 and H9N8 in commercial and rural duck and geese holdings in Northern Italy. In wild waterfowl avian influenza subtypes H1N1 and H10N4 were identified.

Although these infections did not establish themselves in the commercial poultry circuit, it was considered appropriate to introduce vaccination with a bivalent vaccine from 1 October 2004 against both avian influenza subtypes H7 and H5 by Commission Decision 2004/666/EC. Due to the intensive surveillance in the vaccinated and unvaccinated poultry flocks in the area, additional movement restrictions and biosecurity measures with regard to LPAI infected flocks intra-Community trade of chicken/turkey meat and table eggs can be maintained. However, as during all previous vaccination campaigns, intra-Community trade of live vaccinated poultry and hatching eggs derived from vaccinated poultry remains prohibited.

For further update on vaccination against AI in the EU please see:
http://europa.eu.int/comm/food/animal/diseases/controlmeasures/avian/vaccination_en.htm

1.3.1.2. Surveys for AI in poultry and wild birds

By Decision 2004/111/EC (amended by Decision 2004/615/EC) provisions were made to continue surveillance activities for avian influenza subtypes H5 and H7 in poultry and wild birds and to expand them to all 25 Member States (15 old and 10 new Member States after Accession on 1 May 2004).

A total sum of one million Euro was allocated by the Community for co-financing up to 50% of Member States' expenses for the implementation of their surveillance programmes. Financial contributions to the individual programmes were decided by Decision 2004/630/EC (as amended by Decision 2004/679/EC) upon approval of the programmes by the Commission. Payments will be performed after completion of the surveys and final reporting due by March 2005. Following the experiences gained with the previous surveys, guidelines had been revised in order to better target them towards "at risk populations" (e.g. focus on holdings with free range birds). Laboratory testing methods for serological investigations in ducks and geese were further harmonised. For this purpose the CRL has carried out a ring trial involving a restricted number of National laboratories on AI.

1.3.1.3. Review of EU legislation for the control of AI

The state of play for a proposal for the revision of the current Council Directive 92/40/EEC was outlined. The proposal has been discussed in Commission working groups with Member States' experts. The main changes are: to amend the definition to include LPAI subtypes H5 and H7, to enforce the control measures for HPAI, to establish compulsory surveillance and compulsory control measures for LPAI H5/H7, to broaden the possible use of vaccination and to foresee vaccine banks. A flexible approach depending on a risk

assessment of the specific situation is suggested in particular for: the extension of measures to other AI virus subtypes if they pose a major threat, for applying stamping out or controlled slaughter, for controlling infection in "other birds" (such as zoos, wild life parks, rare breeds, endangered species, pet birds, racing pigeons) and for investigations of AI in other species such as pigs. A stakeholder consultation is scheduled for October 2004. In addition, the European Food Safety Authority (EFSA) has been requested for an update of the scientific opinions issued by the former Scientific Committee (SCAHAW) 2000 and 2003 on avian influenza.

1.3.1.4. International trade rules for AI

The World Organisation for Animal Health (OIE) lays down recommendations for trade in live animals and products of animal origin in its Terrestrial Animal Health Code and the Aquatic Health Code in respect to animal diseases.
The new chapter proposed on Avian influenza was not adopted at the OIE 72nd General session in May 2004, but it was included "under study" in the Terrestrial Code 2004. Although there was general agreement of inclusion of LPAI H5 and H7 for recommendations for international trade, no consensus could be reached on the level of surveillance that was needed and on the practical application of compartmentalization.

Official comments of the European Community sent to OIE can be viewed under the following website:
http://europa.eu.int/comm/food/international/organisations/oie_en.htm

1.3.3. NEWCASTLE DISEASE (ND)

1.3.3.1. Finland

Finland (like Sweden, Denmark and Estonia) does not practise prophylactic vaccination against ND in its poultry flocks.
On 19 July 2004 an outbreak of ND in a meat turkey flock in the Southeast of Finland in Satakunta was reported. The flock was destroyed and safely disposed off.

1.3.3.2. Sweden

2 outbreaks of ND in layer flocks located in the province of Östergötland were identified on 20 July 2004. Both flocks had already been culled on suspicion before definite laboratory diagnosis had been available.

1.3.3.2. Control measures for ND in Finland and Sweden

Finland and Sweden applied the control measures as laid down in Council Directive 92/66/EEC by implementing stamping out, movement restrictions on live poultry and poultry products, vehicles, persons etc. and by carrying out epidemiological investigations including testing of possible contact farms.

In both countries outbreaks had been identified due to routine surveillance and no prominent clinical signs were seen. Although ND viruses identified in the Swedish and the Finish outbreaks were essentially identical, no epidemiological link due to movement of birds or by other contacts could be identified. Affected holdings in both countries were located in close proximity to the sea or large lakes; therefore virus introduction via migrating birds seems the most plausible source of infection.

In both countries no further spread of infection occurred and restrictions on intra-Community trade from the affected areas could be lifted on 2 September 2004 (Finland) and on 4 August 2004 (Sweden) respectively, when at least 30 days had elapsed after final cleaning and disinfection of the affected holdings had been carried out under official control.

The Commission kept other Member States and trading partners regularly informed about the disease evolution, the measures taken and the respective trade restrictions. No protection measures were taken at Community level.

1.3.4. Legislation on poultry animal health matters in relation to Accession

1.3.4.1. Contingency plans for AI and ND

According to Directives 92/40/EEC and 92/66/EEC Member States must have contingency plans in place detailing the legal, practical, logistical and financial means to confront AI and ND outbreaks of a major size on their territory. The 10 new Member States therefore had to submit their national contingency plans for AI and ND before Accession in order to have them approved when joining the Community. After evaluation of the plans these have been approved Commission Decision 2004/835/EC.

1.3.4.2. National approval of hatcheries and poultry establishments

According to Council Directive 90/539/EEC on intra-Community trade in and imports of live poultry and hatching eggs Member States must have national plans detailing the procedures for the approval of hatcheries and establishments of breeding and productive poultry. These establishments must be under the official control of the competent authorities, comply with certain hygiene and lay-out standards and disease control programmers (Salmonella and Mycoplasma) as detailed in the legislation.

Following examination by the Commission services plans of the new Member States and updated plans of old Member States have been approved by Commission Decision 2004/402/EC.

2. DISEASE SITUATION IN THIRD COUNTRIES AND SAFEGUARD MEASURES TAKEN IN THIS RESPECT

2.1. Avian influenza in Asia

Since January 2004 outbreaks of highly pathogenic avian influenza **H5N1** have occurred in Cambodia, China (incl. HK), Japan, Indonesia, Laos, Thailand, Vietnam, South Korea and of subtype **H7N3** in Pakistan (1 outbreak). In August 2004 outbreaks of avian influenza H5N1 also appeared in Malaysia.

At the time of the meeting 40 cases of human infections with AI were confirmed by the WHO (World Health Organisation) in Vietnam/Thailand, 29 thereof fatal.

After the first reported outbreak in Thailand - is a major exporter of fresh poultry meat to the EU-imports of fresh poultry meat were banned by Decision 2004/84/EC.

These safeguard measures were prolonged and extended to all other AI affected countries by Decision 2004/122/EC, which has been amended several times as the disease situation evolved. Import restrictions also cover live captive birds, fresh meat of poultry and ratites/farmed and wild feathered game, table eggs, meat preparations, meat products unless heat 70° throughout the product, feed materials containing parts of avian species, untreated game trophies an unprocessed feathers.

The chronology of events and Community action in order to protect animal and human health can be viewed on the following website:
http://europa.eu.int/comm/food/animal/diseases/controlmeasures/avian/index_en.htm
http://europa.eu.int/comm/health/ph_threats/com/Influenza/avian_influenza_en.htm

2.2. Avian Influenza in the United States of America

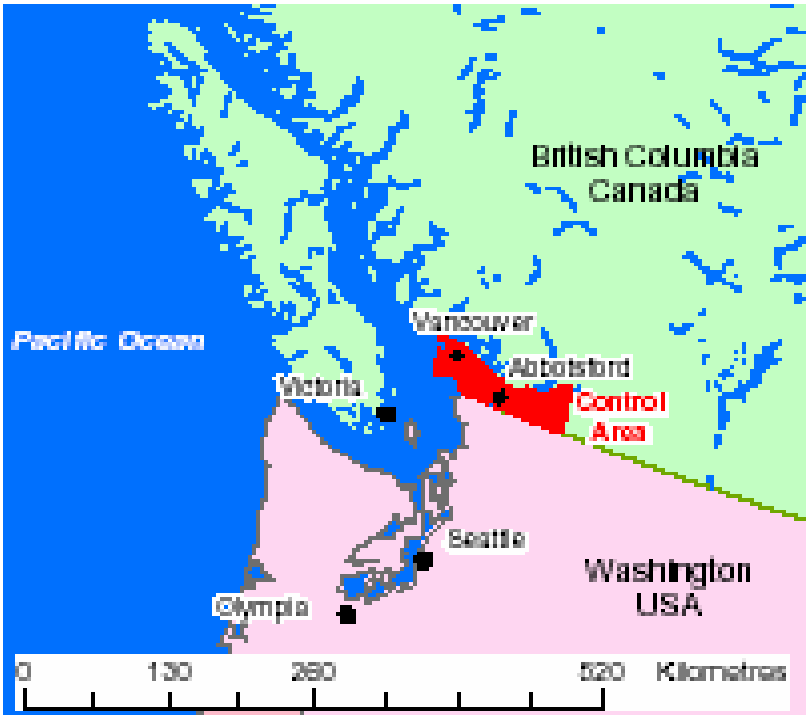
On 23 February 2004 the veterinary administration of the USA reported an outbreak of avian influenza **subtype H5N2** in a broiler holding (~ 6000 birds) in Gonzales county and 2 live bird markets in Houston, Texas (for details of this outbreak see contribution by Dr. Dennis Senne).

EC trade restrictions on live poultry and poultry products were adopted for the whole territory of the United States of America by Commission Decision 2004/187/EC of 24 February 2004. After having provided further information on the disease control measures taken, the results of epidemiological follow-up investigations and evidence that the infection had not spread Decision 2004/363/EC was adopted on 6 April 2004 limiting the trade restrictions to the State of Texas (regionalisation). The remaining restrictions elapsed on 23 August 2004.

The chronology of events the EC measures taken can be viewed on: http://europa.eu.int/comm/food/animal/diseases/controlmeasures/avian/index_en.htm

2.3. Avian Influenza in Canada

On 9 March 2004 CFIA (Canadian Food Inspection Agency) reported an outbreak of avian influenza **subtype H7N3** in a broiler breeder flock in Fraser Valley, in the province of British Columbia. Fraser Valley is a densely populated poultry producing area mainly for the local supply for Vancouver and bordering areas of the USA. On 11 March 2004 EC restrictions for the importation of live poultry and poultry products from the whole territory of Canada were adopted by Commission Decision 2004/242/EC. By the end of May 2004 52 outbreaks were confirmed in 41 commercial flocks and in 11 backyard flocks. 1,2 million birds were reported as infected and depopulation of 16 million birds in the area was performed either by stamping out or by applying pre-emptive slaughter after testing negative for AI. Restocking began on 9 July 2004. By Decision 2004/364/EC restrictions were limited to the established control area in British Colombia (regionalisation). An FVO mission to Canada was carried out in July 2004 in order to asses the disease control measures taken and the guarantees in relation to regionalisation. The restrictions elapse on 1 October 2004.



The chronology of events, the EC measures taken and the FVO mission report can be viewed on: http://europa.eu.int/comm/food/animal/diseases/controlmeasures/avian/index_en.htm and

http://europa.eu.int/comm/food/fvo/ir_search_en.cfm (reference mission number 7323/2004)

2.4. Avian Influenza in South Africa

Since 9 August 2004 outbreaks of avian influenza subtype H5N2 have been reported in at least 5 ostrich farms in the Eastern Cape Province. So far > 13.000 birds have been killed.

EC restrictions on the importation of live ratites and fresh ratite meat (other poultry species and products thereof are not authorised for importation to the EU) from the whole territory of South Africa have been adopted by Decisions 2004/594/EC and 2004/614/EC.

For the chronology of events and EC measures please visit: http://europa.eu.int/comm/food/animal/diseases/controlmeasures/avian/index_en.htm

National Animal Health Laboratory Network (NAHLN)

Dennis A. Senne
Diagnostic Virology Laboratory

National Veterinary Services Laboratories
Veterinary Services, APHIS
Ames, IA, 50010
www.aphis.usda.gov/vs/nvsl

Safeguarding Animal Health

Veterinary Services Managers' Conference, August 26, 2003

The NAHLN – What is it?

- "A network of Federal and State resources, to enhance detection of and enable a rapid and sufficient response to animal health emergencies, including bioterrorism events and foreign animal disease (FAD) outbreaks."
- Three components
  - Surveillance
  - Early Detection
  - Response & Recovery

Safeguarding Animal Health

NAHLN – Surveillance Needs

- Reduce time for outbreak detection
  - Use of rapid screening assays: real-time PCRs
- Demonstrate absence of disease (post-outbreak)
  - Provide assurance to trading partners
- Surveillance testing allows State labs to maintain testing expertise

Veterinary Services
National Veterinary Services Laboratories

Safeguarding Animal Health

NAHLN - Detection

- Real-time PCRs considered screening tests – positives considered “suspect”
- The NVSL still makes official first diagnosis of FADs in a new region

Veterinary Services
National Veterinary Services Laboratories

Safeguarding Animal Health

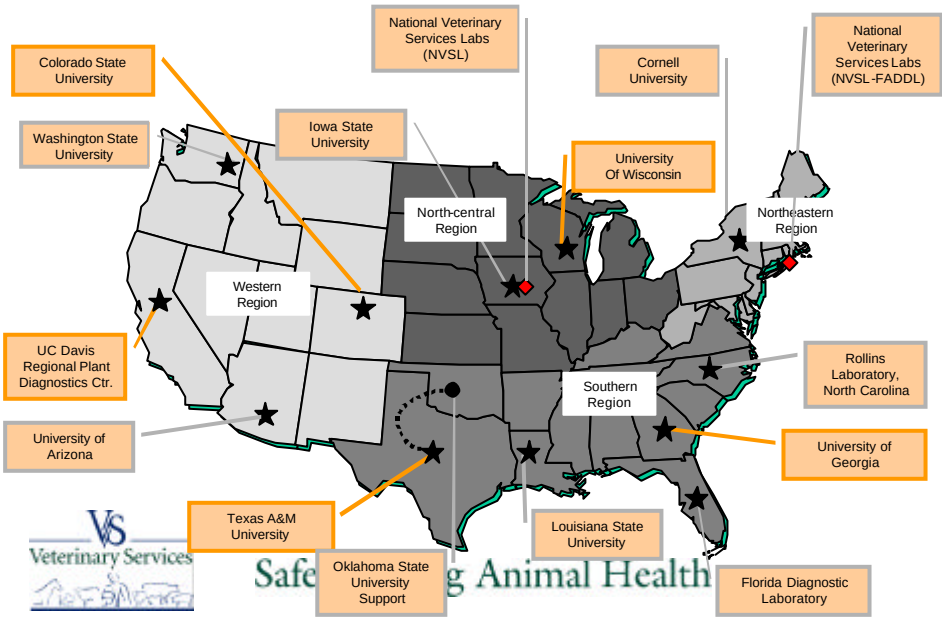
NAHLN – Response & Recovery

- Reduce response time in face of outbreak
- Quickly define extent of the outbreak and prevent spread
- Federal labs may be overwhelmed by the large number of samples which must be run quickly
- State labs could serve as “surge capacity” for federal labs and each other
- Decrease distance from outbreak to lab

Veterinary Services
National Veterinary Services Laboratories

Safeguarding Animal Health

Pilot National Animal Health Laboratory Network



Pilot NAHLN – 8 OIE List A Diseases

High Consequence Livestock Pathogens – nonzoonotic

- Avian Influenza (highly pathogenic)
- Exotic Newcastle Disease
- African Swine Fever
- Classical Swine Fever
- Contagious Bovine Pleuropneumonia
- Foot-and Mouth Disease
- Lumpy Skin Disease
- Rinderpest

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National Veterinary Services Laboratories

Safeguarding Animal Health

Assays – Timetable

- Real-time PCR Assays for 8 FADs - Deployment
  - '03 – HPAI (02), END
  - '04 – CSF, FMD (end of '04)
  - '05 – ASF
  - '06 – Rinderpest, Lumpy Skin Disease
  - '0? – Contagious Bovine Pleuropneumonia

Veterinary Services
National Veterinary Services Laboratories

Safeguarding Animal Health

Features of the NAHLN

- Standardized, rapid diagnostic techniques
- Trained personnel, modern equipment
- Quality standards, proficiency testing
- Adequate facility biosafety/biosecurity levels
- Secure communication, alert, reporting system
- Scenario testing

Veterinary Services
National Veterinary Services Laboratories

Safeguarding Animal Health

Role of NVSL – Developmental Studies and Lab Monitoring

- Development and validation of new diagnostic procedures
  - RRT-PCR tests for AI and ND
  - Used in recent outbreaks – AI (VA, TX), ND (CA)
- NAHLN labs – PCR training
  - 7 persons in 25 labs rec'd training
  - Proficiency tests to >70 persons (35 labs)

Veterinary Services

Safeguarding Animal Health

2002/2003 vND Outbreak: *Affected States, Regions*

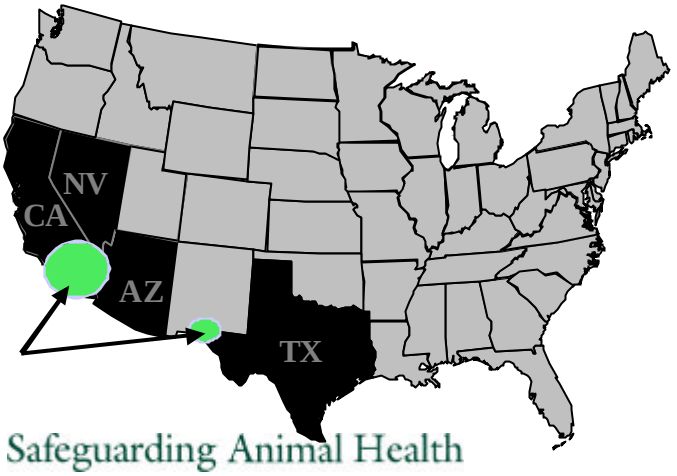
California (CA)

Nevada (NV)

Arizona (AZ)

Texas (TX)

Affected Areas



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2002/2003 END Outbreak: (*CA, NV, AZ, TX)*

Backyard Flocks

- 2,671 premises positive or DC
- 149,247 birds depopulated
- 19,056 premises quarantined
- 200,000 premises surveyed

Commercial Flocks

- 21 premises positive (all in CA)
- >3.2 million birds depopulated

Veterinary Services

Safeguarding Animal Health

Laboratory Diagnosis (ND)

- Rapid diagnostics developed during outbreak
- Real Time RT-PCR (RRT-PCR)
  - Matrix primers/probe – all APMV-1 strains
    - DxSN = 96.7%, DxSP = 97.3%
  - Cal/Mex primers/probe – virulent NDV
    - DxSN = 92.9%, DxSP = 99.1%
  - Avirulent primers/probe – vaccine strains
    - (validation pending)

Veterinary Services

Safeguarding Animal Health

Laboratory Capacity (ND Outbreak)

- RRT-PCR replaced virus isolation – April 22
- Over 100,000 RRT-PCR tests performed between April 22 and Sept 15, 2003 (83,000 done at CDFA)
- High throughput methed – tested between 1,380-1,932 samples/day
  - 3 technicians
  - 3 cyclers (96 well format)
- Single tube format – 184 samples/day

Veterinary Services

Safeguarding Animal Health

HPAI H5N2 - Texas

Veterinary Services

Safeguarding Animal Health

HPAI H5N2 – Texas (2004)

- Real Time RT-PCR (RRT-PCR)
- Developed in 2001 (additional validation – VA LPAI H7N2 2002)
  - Matrix primers/probe – all subtypes of AIV
    - SPECIMEN: DxSN = 88.2%, DxSP = 99.5%
    - SUBMISSION: DxSN = 95.1%, DxSP = 99.1%
  - H5 primers/probe – North American H5 strains
  - H7primers/probe – North Amereican H7 strains

Veterinary Services

Safeguarding Animal Health

Surveillance: US/Canada Boarder-2004

- Response to HPAI H7N3 in Canada
- AI RRT-PCR – 1,621 samples
  - 966 WSU Puyallup
  - 655 WSU-Pullman
- AGID AI = 2,863
- Virology AI = 222

Veterinary Services

Safeguarding Animal Health

Summary

- NAHLN – new concept
- Successfully utilized in recent outbreaks
- More FAD diagnostic responsibility to States with Federal oversight
- Greatly increases laboratory capacity to respond to FADs

Veterinary Services

Safeguarding Animal Health

Immunoselection and characterisation of attenuated NDV strains suitable for *in ovo* vaccination

Jan Mast, Cécile Nanbru, Bénédicte Lambrecht,
Guy Meulemans & Thierry van den Berg

Weybridge, Octobre 1, 2004

"*In ovo* vaccination"

Opportunity to :

immunise each individual chick

without additional stress of manipulation

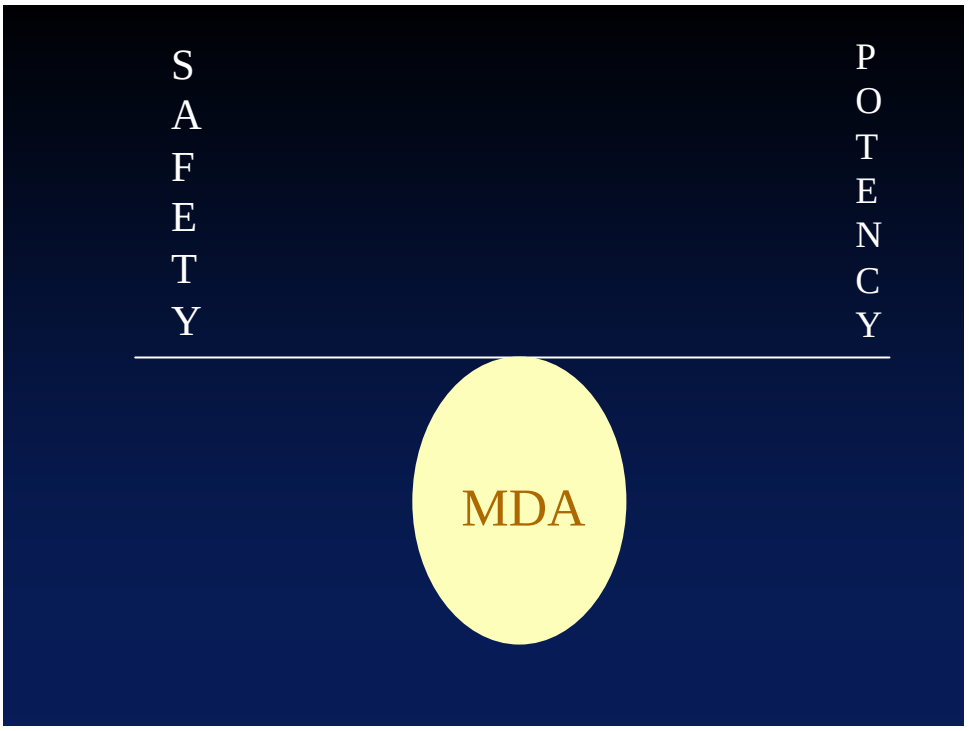
Vaccination *in ovo*: rationale

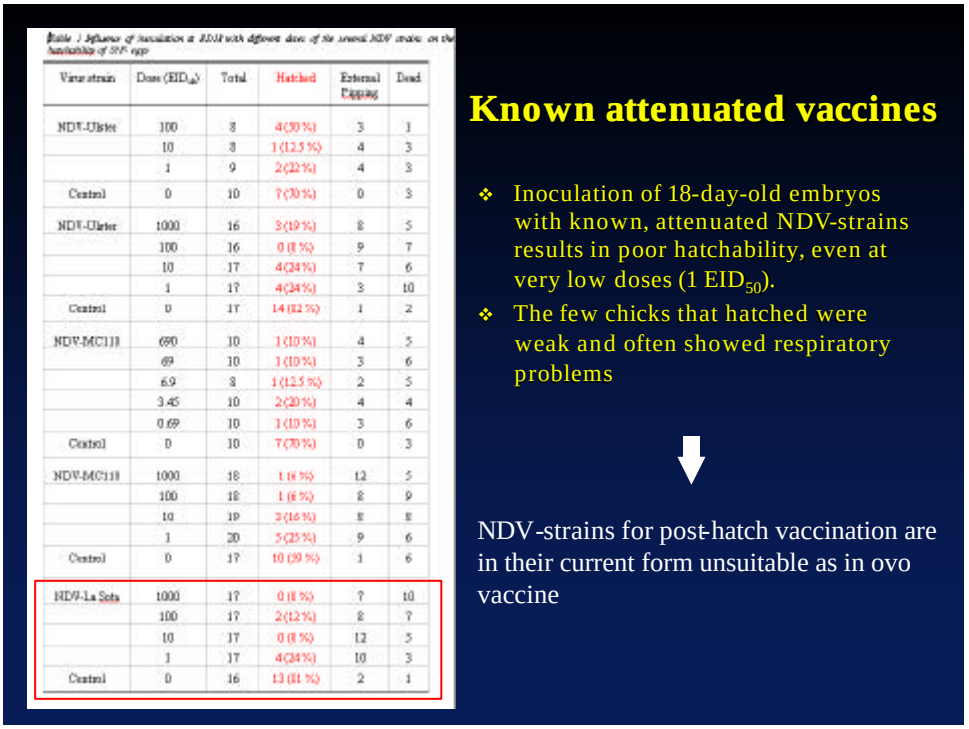
- Rapidly growing poultry industry
  - Labor-saving technologies
  - Improvement in
    - genetic selection
    - management practices
    - Nutrition
    - Disease control
- INOVOJECT:
  - Inoculates between 20.000 & 30.000 eggs per hour
  - Performs egg transfer
  - Eliminates need of post-hatch inoculation
- Reason : better controlled and less expensive

Vaccination *in ovo*: practice

Not all vaccines can be used

- So far, commercial *in ovo* vaccines are only available (registered) against
  - Marek 's disease
  - Infectious bursal disease (IBD, Gumboro) = Icx vaccines
- Newcastle disease virus: experimental phase
  - NDV-strains for post-hatch vaccination are in their current form unsuitable as *in ovo* vaccine due to their residual pathogenicity



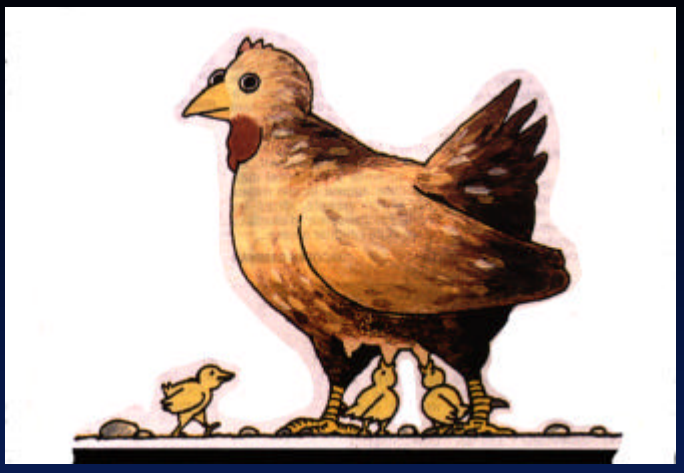


Virus strain	Dose (EID_{50})	Total	Hatched	External Pipping	Dead
NDV-Ulster	100	8	4 (50 %)	3	1
	10	8	1 (12.5 %)	4	3
	1	9	2 (22 %)	4	3
Control	0	10	7 (70 %)	0	3
NDV-Ulster	1000	16	3 (19 %)	8	5
	100	16	0 (0 %)	9	7
	10	17	4 (24 %)	7	6
	1	17	4 (24 %)	3	10
Control	0	17	14 (82 %)	1	2
NDV-MC110	690	10	1 (10 %)	4	5
	69	10	1 (10 %)	3	6
	6.9	8	1 (12.5 %)	2	5
	3.45	10	2 (20 %)	4	4
	0.69	10	1 (10 %)	3	6
Control	0	10	7 (70 %)	0	3
NDV-MC110	1000	18	1 (6 %)	12	5
	100	18	1 (6 %)	8	9
	10	19	3 (16 %)	8	8
	1	20	5 (25 %)	9	6
Control	0	17	10 (59 %)	1	6
NDV-La Sota	1000	17	0 (0 %)	7	10
	100	17	2 (12 %)	8	7
	10	17	0 (0 %)	12	5
	1	17	4 (24 %)	10	3
Control	0	16	13 (81 %)	2	1

Known attenuated vaccines

- Inoculation of 18-day-old embryos with known, attenuated NDV-strains results in poor hatchability, even at very low doses (1 EID_{50}).
- The few chicks that hatched were weak and often showed respiratory problems

NDV-strains for post-hatch vaccination are in their current form unsuitable as in ovo vaccine

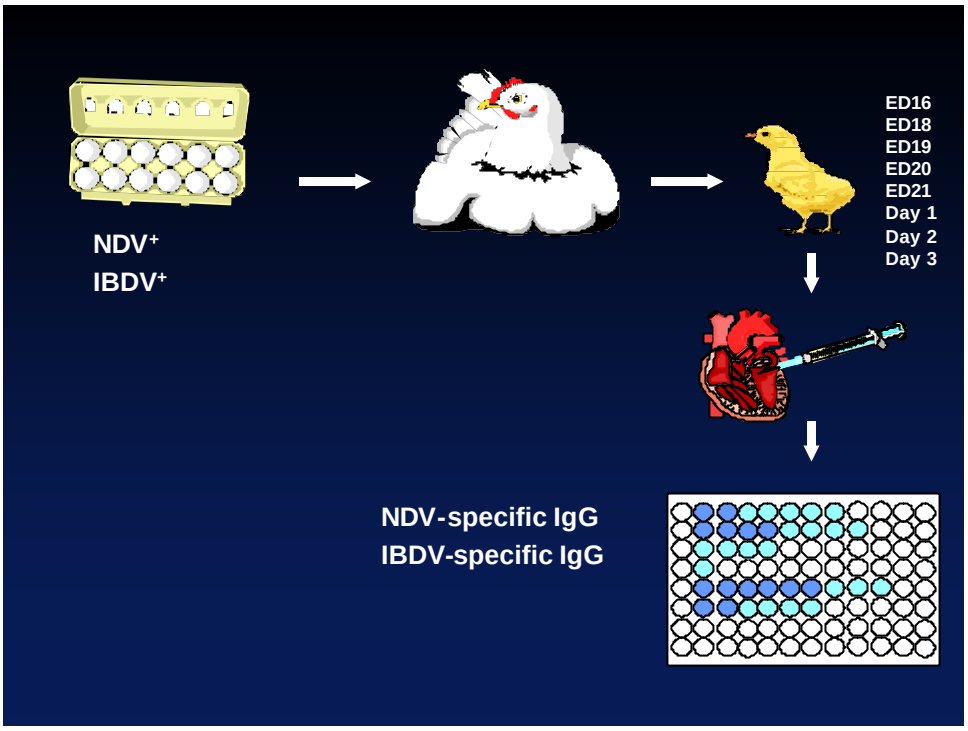


The interference of Maternally Derived Antibody

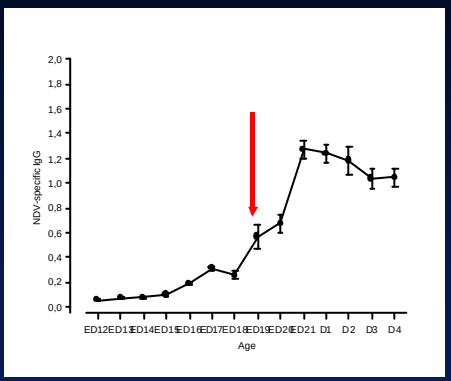
Maternal antibodies and *in ovo* Vaccination

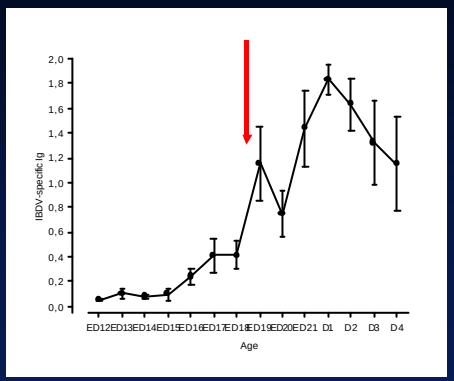
- **Protection against pathogens in the neonatal stage, when immunocompetence is not yet completely developed**
- **Maternal antibodies of birds are stored in the yolk sac and transferred to the embryo while it matures**
- **Interfer with vaccination by specifically eliminating vaccine, so that the optimal vaccination dose is dependent on the titre of MDA**

Analysis of the kinetics of the transfer of maternal antibodies from the yolk sac to the serum



Transfer of maternal antibodies from the yolk sac to the chicken

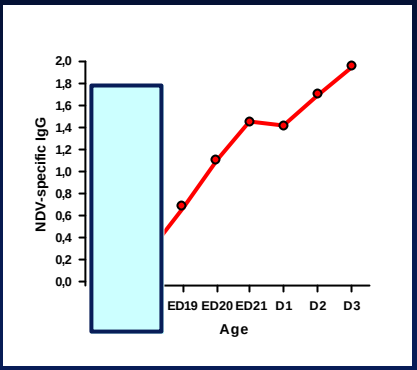




"A window of opportunity exists for avoiding interference of maternal antibodies with vaccination"

In ovo vaccination:

- **low titres of maternal antibodies**
- **virus replication remains possible**
- **low vaccination doses evoke strong, long-lasting immune responses**

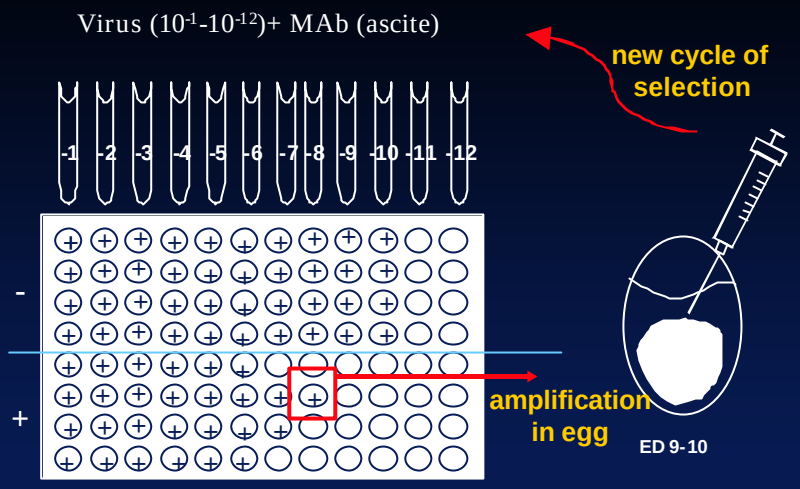


Mutated NDV-strains with reduced virulence

- Meulemans et al. (1987) selected HN and F antigenic variant viruses from the velogenic Italian NDV strain using MAbs directed against these two viral glycoproteins.
- The immunoselected viruses could be regarded as true variant viruses on the basis of cross neutralisation results.
- Some of these variant viruses showed lower IVPI than the parental Italian virus strain.

- Can variants be obtained from the lentogenic La Sota strain using the same MAb that yielded less virulent, but still velogenic, Italian strain viruses ?
- Will these mutant La Sota viruses be less pathogenic for chicken embryos than the parental La Sota strain ?

Immunoselection procedure



The selected isolate is « cycled » 3 or 4 times until titre with or without Mo Ab is ~ identical (< 1 log difference)

Mutated NDV-strains with reduced virulence

- **HN and F antigenic variants could be obtained from La Sota, by immunoselection using specific MAb**
- **These were true variant viruses as their titer after neutralisation with the homologous MAb differed with less than 1 log 10 from the untreated control**
- **Haemagglutination of the HN mutant was not inhibited by many HN-specific MAb**

Less pathogenic for embryos ?

Influence of inoculation at ED18 with different doses of the NDV-La Sota HN-mutant strain (Exp. 1-3) and the F-mutant strain (Exp. 4) on the hatchability and neonatal survival in SPF chicks

Experiment	Dose (EID_{50})	Eggs	Hatched	Neonatal survival (10 d)	Global survival (10 d)
1 (HN-mutant)	100000	18	3 (17 %)		
	10000	18	2 (11 %)		
	1000	17	3 (18 %)		
	100	17	8 (47 %)		
	0	21	11 (52 %)		
2 (HN-mutant)	1000	21	16(76 %)	10/16 (62 %)	48 %
	100	21	5 (24 %)	3/5 (60 %)	14 %
	10	21	15 (71 %)	13/15 (87 %)	62 %
	1	20	11 (55 %)	10/11 (91 %)	50 %
	0	21	13 (62 %)	10/13 (77 %)	47 %
3 (HN-mutant)	200	18	11 (61 %)	10/11 (91 %)	55 %
	100	18	16 (89 %)	16/16 (100 %)	89 %
	50	18	13 (72 %)	13/13 (100 %)	72 %
	25	18	16 (89 %)	16/16 (100 %)	89 %
	12.5	18	14 (78 %)	10/13 (77 %)	55 %
	0	18	15 (83 %)	15/15 (100 %)	83 %
4 (F-mutant)	1000	15	10 (%)	3/10 (30 %)	20 %
	100	15	7 (%)	3/7(43 %)	20 %
	10	15	11 (%)	4/11(36 %)	27 %
	1	15	8 (%)	4/8(50 %)	27 %
	0	15	12 (%)	10/12 (83 %)	67 %

Mutated NDV-strains with reduced virulence

- The pathogenicity of both the HN- and the F-mutant for embryos and young chicks was reduced substantially in comparison with the parental La Sota strain
- F and HN are responsible for different physiological functions.

Can the pathogenicity be further reduced by the introduction of a mutation in both genes simultaneously, obtaining so-called double mutants ?

Selection of double escape mutants

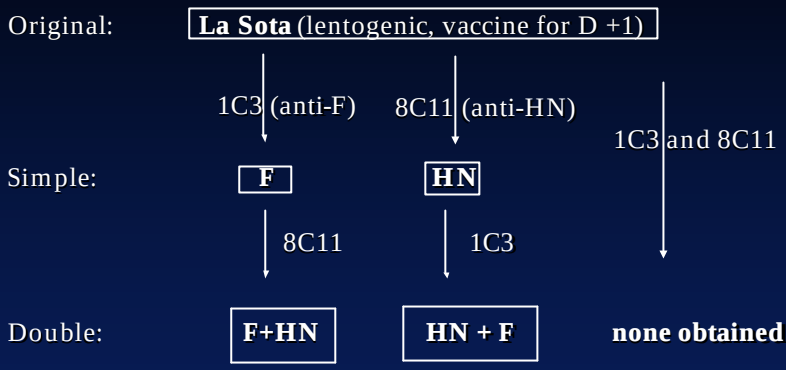


Table 1 Neutralisation of antigenic variants after neutralisation using homologous MAb

	Virus titre after passage (EID_{50}/ml)				
	Treatment	1st passage[a]	2nd passage	3rd passage	4th passage
F mutant	MAb 1C3	$2.13 * 10^4$	$> 1.58 * 10^9$	$1.20 * 10^9$	$1.58 * 10^8$
	No MAb	$> 1.58 * 10^9$	$> 1.58 * 10^9$	$2.39 * 10^9$	$2.13 * 10^9$
HN mutant	MAb 8C11	$1.58 * 10^6$	$1.20 * 10^6$	$1.58 * 10^7$	$1.58 * 10^6$
	No MAb	$> 1.58 * 10^9$	$> 1.58 * 10^9$	$2.39 * 10^8$	$5 * 10^7$
F+HN mutant	MAb 8C11	$2.13 * 10^5$	$3.38 * 10^5$	$9.96 * 10^8$	
	No MAb	$1.58 * 10^9$	$3.38 * 10^8$	$5 * 10^8$	
HN+F mutant	MAb 1C3	$1.58 * 10^2$	$7.39 * 10^7$	$2.13 * 10^7$	
	No MAb	$1.58 * 10^8$	$2.39 * 10^8$	$7.39 * 10^7$	

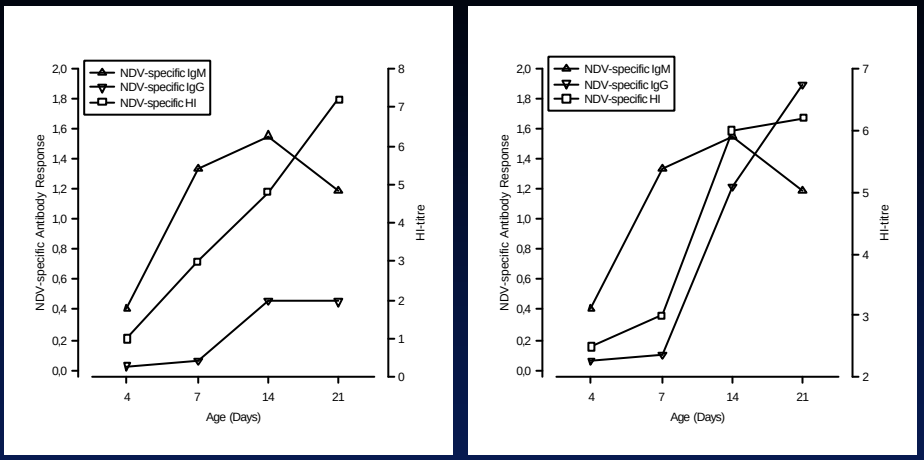
[a] The virus titer of the parental NDV La Sota strain was $1.58 * 10^{10}$ EID50/ml).

Less pathogenic for embryos ?

Influence of inoculation at ED18 with different doses of the NDV-La Sota F+HN strain on the hatchability and neonatal survival in SPF chickens

NDV strain [a]	Dose (EID_{50})	Eggs	Hatched	Neonatal survival (10 d)	Global survival (10 d)
La Sota	10^3	17	0 (0 %)	N.D. [b]	N.D.
	10^2	17	2 (12 %)	N.D.	N.D.
	10	17	0 (0 %)	N.D.	N.D.
	1	17	4 (24 %)	N.D.	N.D.
	0	16	13 (81 %)	N.D.	N.D.
HN mutant	10^3	21	16(76 %)	10/16 (62 %)	48 %
	10^2	21	5 (24 %)	3/5 (60 %)	14 %
	10	21	15 (71 %)	13/15 (87 %)	62 %
	1	20	11 (55 %)	10/11 (91 %)	50 %
	0	21	13 (62 %)	10/13 (77 %)	47 %
F mutant	10^3	15	10 (67 %)	3/10 (30 %)	20 %
	10^2	15	7 (47 %)	3/7(43 %)	20 %
	10	15	11 (73 %)	4/11(36 %)	27 %
	1	15	8 (53 %)	4/8(50 %)	27 %
	0	15	12 (80 %)	10/12 (83 %)	67 %
HN+F mutant	10^6	12	5 (42 %)	1/5 (20 %)	8 %
	10^5	12	5 (42 %)	3/5 (60 %)	25 %
	10^4	12	4 (25 %)	2/4 (50 %)	16 %
	10^3	12	10 (83 %)	8/10 (80 %)	67 %
	0	14	12 (86 %)	11/12 (92 %)	92 %
F+HN mutant	10^6	12	7 (58 %)	4/7 (57 %)	33 %
	10^5	12	11 (92 %)	7/11 (64 %)	58 %
	10^4	12	9 (75 %)	7/9 (78 %)	58 %
	10^3	12	12 (100 %)	10/12 (83 %)	83 %
	0	14	12 (86 %)	11/12 (92 %)	92 %

a Most significant results of three (HN mutant), two (HN+F mutant) and four experiments (F+HN mutant)



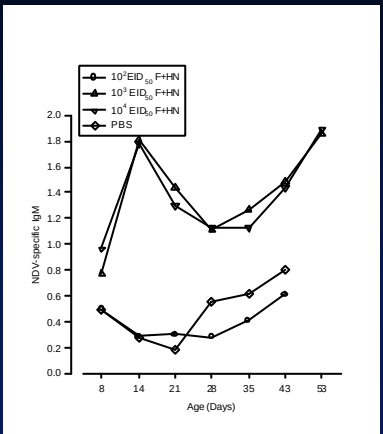
Mean NDV-specific responses upon vaccination with 10^3 EID_{50} (left, experiment 1 or right, experiment 5) of the La Sota F+HN-mutant in function of age in SPF chickens.

In ovo vaccination of commercial broilers : Hatchability

Mutant virus strain	Dose (EID_{50})	Total	Hatched	Unexplained post-hatch mortality
(F+HN)	10^4	51	46 (90 %)[a]	6/46 [a]
	10^3	51	41 (80 %)	1
	10^2	51	41 (80 %)	0
Control	0	51	42 (82 %)	1

a Mortality was observed from D10 onwards. No clinical signs were observed, except feather picking.

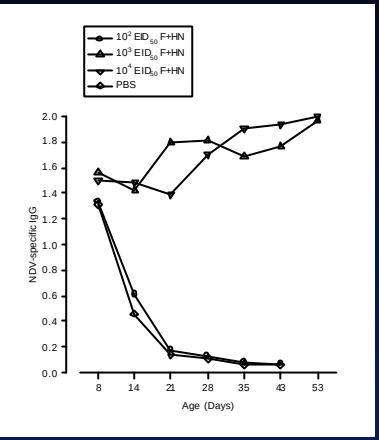
In ovo vaccination of commercial broilers : NDV-specific IgM



- 10^3 and 10^4 EID_{50} : NDV-specific IgM response, peaking around 14 days
- PBS-treated and 10^2 EID_{50} : No NDV-specific IgM response

For low doses, humoral responses are dependent of maternal antibodies

In ovo vaccination of commercial broilers : NDV-specific IgG



- 10^3 and 10^4 EID_{50} : NDV-specific IgG increase gradually with age indicating an active IgG-respons
- PBS-treated and 10^2 EID_{50} : NDV-specific maternal IgG decrease gradually

For adequate vaccine doses, seroconversion is induced

In ovo vaccination of commercial broilers : Virus transmission

- NDV-specific IgM, IgG and HI-responses in 14- and 21-day-old SPF chicks (sentinels) housed together with chicks vaccinated in ovo with 10^3 en 10^4 EID_{50} F+HN
- No humoral NDV-specific responses in 14- and 21-day-old sentinels housed together with unvaccinated chicks or chicks vaccinated in ovo with 10^2 EID_{50} F+HN

If adequate doses are used, the vaccine virus

- proliferates in the presence of maternal antibodies
- is transmitted from vaccinated to non-vaccinated chicks

In ovo vaccination of commercial broilers : Protection upon challenge

Treatment	Cumulative mortality on day 53	
	n	%
PBS-treated	11/12 [a]	92
10^2 EID_{50} F+HN	12/12	100
10^3 EID_{50} F+HN	0/12	0
10^4 EID_{50} F+HN	0/9	0

[a] The surviving chick was moribund because of paralysis of its limbs

Characterisation of NDV-mutants

Level	Information	Method
Genomic	RNA sequence	Sequencing of HN and F genes
Protein	Expression of epitopes	Indirect ELISA
Function	(Haem)adsorption	HI (MAb)
	Neuraminidase	Colorimetric assay for NA
	In ovo virulence	Hatchability, neonatal survival

Summary of results

	La Sota	F	HN	F+HN	HN+F
Epitope lost by ELISA	/	1C3, 10F2	/	1C3, 10F2, 8C11	1C3, 10F2
Epitope lost by HI	/	/	8C11, 4D6, partial 6C6, 7B7, 7D4, 5A1	8C11	8C11
Mutation F (aa position)	/	72		72	72
Mutation HN (aa position)	/		193	229	193, 160
Neuraminidase activity (100%)	100	107	106	40	56

Conclusions and Perspectives

- **EFFICACY of La Sota F+HN mutant for *in ovo* vaccination (protection of vaccinated chicks)**
- **RESIDUAL PATHOGENICITY in SPF chicks requirement in USA and Europe is a ten time release dose safety test in SPF chicks**
  - Reduce pathogenicity by immune-complex formation
  - Further attenuation by creating triple mutants from F+HN
- **SAFETY in conventional chickens**
- **Marker vaccine :**
  - Serology → competitive ELISA
  - Virus isolation → HAI tests with Mabs
  - Virus detection by RT-PCR → sequencing

PATHOGENICITY OF NDV STRAINS ISOLATED FROM PIGEONS IN POLAND

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Newcastle disease (ND) in pigeons, caused by the "pigeon variant" of paramyxovirus serotype 1 (PPMV-1), was first described in the Middle East at the end of the 1970s (7) and spread rapidly throughout the world (3). In Poland, first cases of "pigeon paramyxovirosis" were recognized in 1983 (9). However, first isolation and preliminary characterization of PPMV-1 was carried out at the end of 1980s (10). PPMV-1 viruses are pathogenic for pigeons, but chickens can also be affected (1,2).

According to OIE definition for the purpose of trade, control measures and policies (8), Newcastle Disease is an infection of birds caused by avian paramyxovirus serotype 1 which has an intracerebral pathogenicity index (ICPI) in day-old chicks of 0.7 or greater or presence of multiple basic amino acids has been demonstrated at the cleavage site of F protein.

The aim of the study was identification and assessment of pathogenicity of PPMV-1 strains isolated from pigeons in Poland by *in vivo* and *in vitro* methods.

Materials and methods

Virus isolates. Eleven PPMV-1 strains were used: eight strains isolated form racing pigeons at the end of 80-ties (early strains) and three strains isolated from feral pigeons in 2002 (recent strains). Lentogenic NDV La Sota and velogenic PPMV-1 Italy strains were used for comparison. The strains were propagated on SPF embryonated eggs (Valo-Lohmann), allantoic fluids were harvested and used for further study.

Serological identification. The allantoic fluids exhibiting haemagglutination activity (HA) were tested in haemagglutination inhibition test (HI) using NDV polyclonal antiserum and monoclonal antibodies 7D4 (specific for La Sota strain) and Mab 161 (specific for PPMV-1) kindly provided by VLA Weybridge, UK. HI test was performed according to the Annex III of Council Directive 92/66/EEC (4)

RNA extraction and reverse-transcription polymerase chain reaction. RNA was extracted from allantoic fluids with HA activity using commercial test (Qiagen®). Reverse transcription (RT) was performed for 50 min. in 42ºC in a total volume of 20 µl containing 5µl RNA, 0,1 µg of hexamers, 200 µM of dNTP, 4 µl of reaction buffer (5x), 0,1 M DTT, 20U of ribonuclease inhibitor, 200U reverse transcriptase (Invitrogen, USA). For polymerase chain reaction (PCR), primers to amplify the region of fusion protein (F) gene containing cleavage site were described by Creelan *et al.* (5). PCR was carried out in a total volume of 50 µl containing 5 µl of cDNA, 5µl of PCR buffer 10x, 1 µl of dNTPs (25 mM each), 4 µl of $MgCl_2$ (25 mM), 1,5 Taq polymerase (Fermentas, Lithuania) and 2,5 µl of primers. The amplification was performed as follows: 94^0C – 2 min (initial

denaturation), 94^{0}C – 15 sec (denaturation), 48^{0}C – 30 sec (annealing), 70^{0}C – 30 sec (elongation) x 40 followed by 70^{0}C -7 min (final elongation). The PCR products (expected size 202 bp) were separated on 1,5 % agarose gel.

Restriction enzyme analysis. PCR products were cleaved by *Bgl*I enzyme in a final volume of 20 µl according to manufacturer's instruction (MBI, Fermentas, Lithuania). Incubation was performed overnight in 37 ^{0}C. Separation was carried out on 3% agarose gel.

Sequencing. PCR products were sent for sequencing to the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw. The sequences were aligned and amino acids predicted using GeneDoc Multiple Sequence Alignement (Editor & Shading Utility program).

Pathogenicity in vivo. ICPI was determined on one-day old SPF chicks according to the Annex III of Council Directive 92/66/EEC (4).

Results and discussion

Results are shown in the Table 1. All isolates revealed positive reaction with NDV antiserum and Mab 167, which recognizes "pigeon variants" of paramyxoviruses serotype 1, and therefore have been classified as PPMV-1. The results were confirmed by RT-PCR. The ICPI value was greater than 0,7 (9 strains) or below 0,7 (2 strains) and it was generally higher in early strains (mean 1,2) than in recent strains (mean 0,59). However, following restriction enzyme analysis (REA) using *Bgl*I, PCR products of all Polish isolates were not digested while LaSota strain, used for comparative studies, was cleaved into two bands (135 bp and 67 bp). It was shown by Creelan *et al.*(5), that *Bgl*I enzyme cleaves PCR products of lentogenic strains while cDNA of mesogenic and velogenic strains remain uncleaved. Restriction profile of all examined Polish isolates was typical for virulent strains. Three different motifs in the cleavage site of F protein were found among early PPMV-1 strains (so far the sequence of 7 strains has been determined): ^{112}GRQKRF^{117}, ^{112}RRQKRF^{117}, ^{112}RRKKRF^{117} whereas recent strains isolated in 2002 possessed a motif ^{112}RRQKRF^{117}. All these sequences fulfilled the criteria established in OIE for virulent strains. Moreover, two PPMV-1 strains with the ICPI <0,7 showed clearly virulent molecular pattern. Lack of complete correlation between *in vivo* and *in vitro* tests were described previously by Meulemans *et al.* (6) who demonstrated presence of multiple basic amino acids at the F2/F1 cleavage site in 14 pigeon NDV strains with the ICPI below 0,7. We therefore suggest that RT-PCR and sequencing should be preferred method of the pathogenicity evaluation. REA method can be helpful in preliminary assessment of the virulence.

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Table.1 Characterization of NDV strains isolated from pigeons in Poland

Strain	Identification		Pathogenicity		
	HI	RT-PCR	ICPI	REA	Sequence
AR/1/88	PPMV-1	PMV-1	1,12	not cleaved	^{112}GRQKRF^{117}
AR/2/88	PPMV-1	PMV-1	1,36	not cleaved	^{112}RRQKRF^{117}
AR/3/88	PPMV-1	PMV-1	1,42	not cleaved	^{112}RRQKRF^{117}
AR/4/88	PPMV-1	PMV-1	1,25	not cleaved	nt*
AR/5/88	PPMV-1	PMV-1	1,27	not cleaved	nt
AR/6/88	PPMV-1	PMV-1	1,27	not cleaved	nt
AR/7/88	PPMV-1	PMV-1	1,05	not cleaved	^{112}RRKKRF^{117}
AR/8/88	PPMV-1	PMV-1	0,87	not cleaved	nt
PW/46-55/02	PPMV-1	PMV-1	0,42	not cleaved	^{112}RRQKRF^{117}
PW/56-66/02	PPMV-1	PMV-1	0,75	not cleaved	^{112}RRQKRF^{117}
PW/166-175/02	PPMV-1	PMV-1	0,61	not cleaved	^{112}RRQKRF^{117}
PMV-1 Italy	PPMV-1	PMV-1	1,55	not cleaved	nt
PMV-1La Sota	PMV-1	PMV-1	0,14	cleaved	^{112}GRQGRL^{117}

* not tested

Selection of different pathotypes from a single isolate of PPMV-1

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Outline

- Newcastle disease
- Pigeon panzootic (PPMV-1)
- Background to research
- Selection procedure
- Results

Current OIE definition of Newcastle Disease

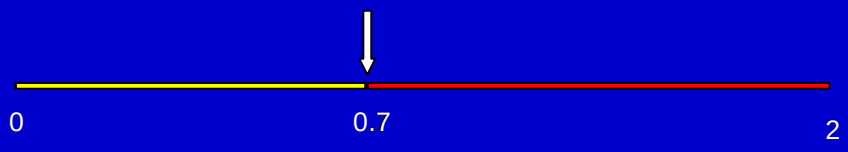
Newcastle disease is an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence:

a) The virus has an intracerebral pathogenicity index (ICPI) in day-old chicks (*Gallus gallus*) of 0.7 or greater.

or

b) Multiple basic amino acids have been demonstrated in the virus at the C-terminus of the F2 protein and phenylalanine at residue 117…The term 'multiple basic amino acids refers to at least three arginine or lysine residues between residues 113 to 116.

Intracerebral Pathogenicity Index



Eg. ^{112}GKQGRL^{117}

Eg. ^{112}RRQKRF^{117}

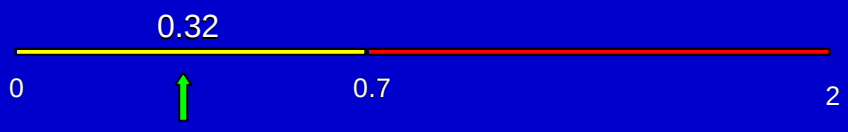
Virus above 0.7 are subject to statutory control

Pigeon Panzootic

- Pandemic of variant NDV spread throughout Europe beginning in the 1980's
- Antigenically and genetically very similar but distinguishable from APMV-1 isolated from poultry
- Responsible for 22 outbreaks in chickens in the UK
- Outbreaks of disease in pheasants, birds of prey, pet birds and some wild birds
- Continues to cause disease in feral, show and racing pigeons

The Basis for this Research

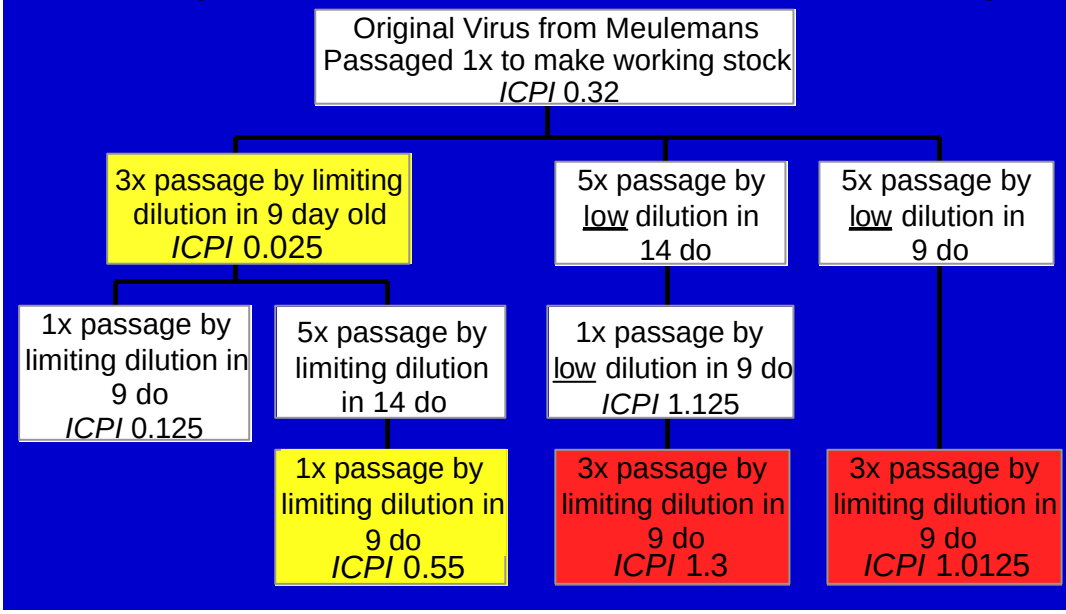
- Some PPMV-1 isolates have displayed unusual pathogenic properties
- Despite having a cleavage site motif characteristic to virulent viruses they exhibit an ICPI < 0.7
- Eg. Meullemans et al. (2002) Belgium PPMV-1



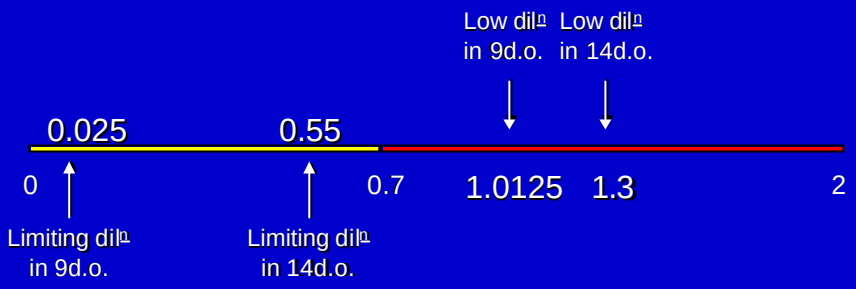
Sequence at the F2/F1 Cleavage site ^{112}RRQKRF^{117}

- The virulence for chickens of PPMV-1 viruses is greatly increased by 3-4 passages in this host
- It has been observed that low pathogenic influenza (with a virulent cleavage motif) on passage in 14-day-old embryos will increase its virulence

Summary of Selection Procedure in Chicken Embryos



The Change In Pathogenicity Of 1073/98 248VB Following Different Passage Procedures



All share the same cleavage site RRQKRF

Sequence differences between the HN and F

- Pathotypes 0.025 and 0.55 are identical
- Pathotypes 1.0125 and 1.3 are also identical
- These two pairs differ by one nucleotide

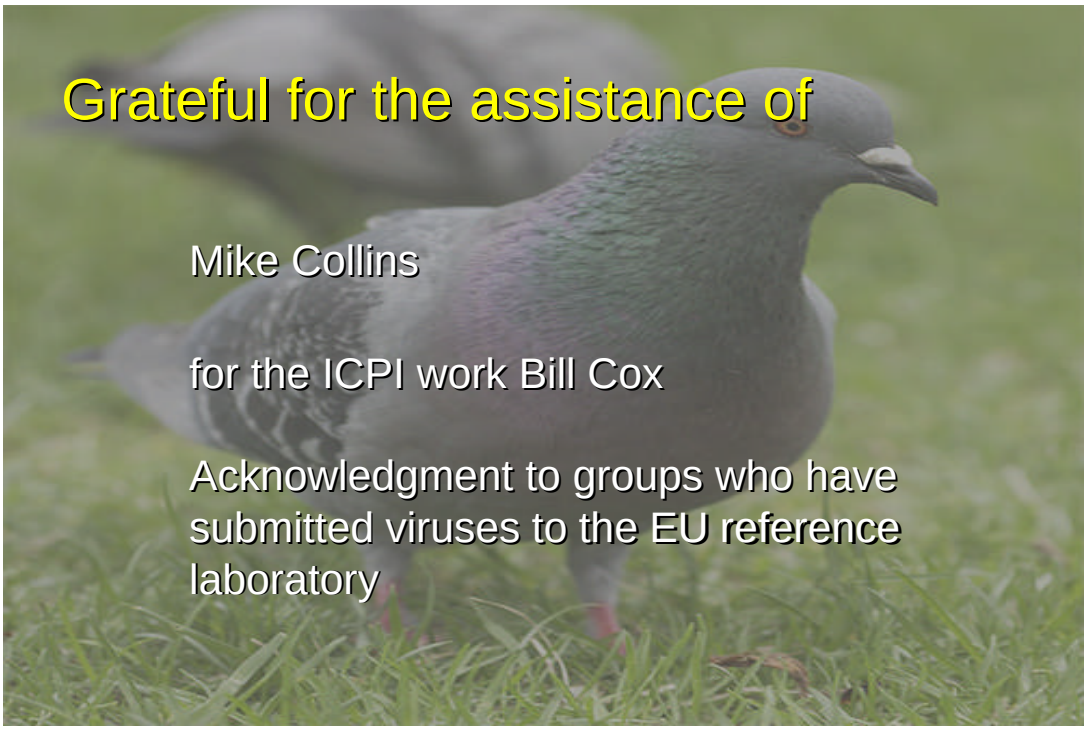
ICPI	nucleotide at position 1403 Of the fusion gene	amino acid at position 453 of the fusion protein
0.025	C	P
0.55	C	P
1.3	T	S
1.0125	T	S

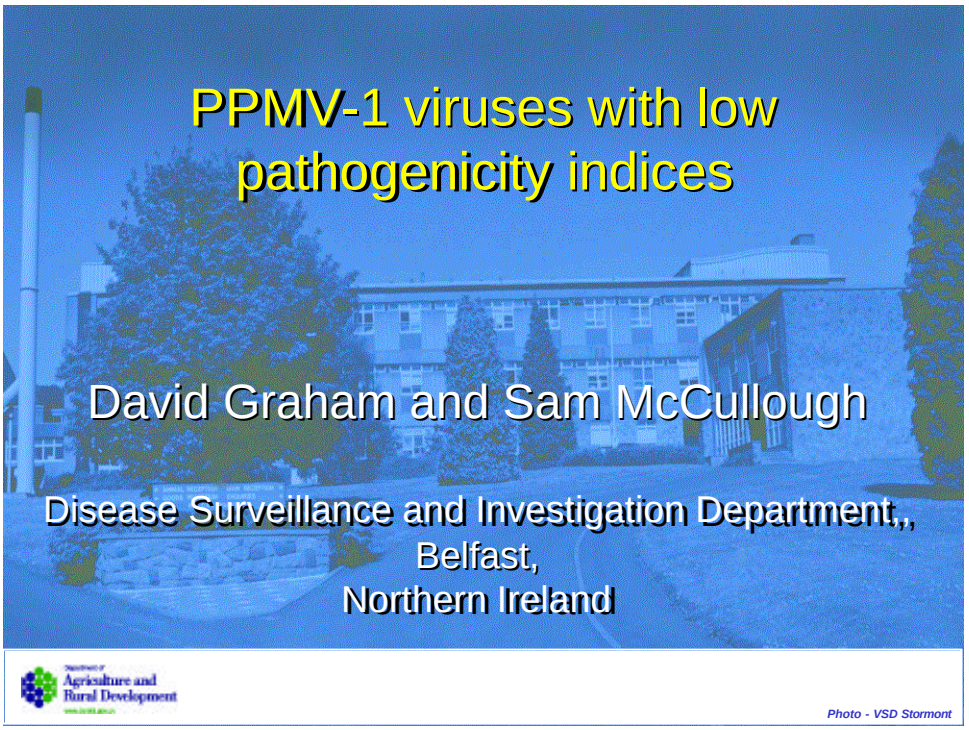
Conclusions

- Some PPMV-1 isolates do not conform
- Selection of different pathotypes from a single isolate
- Genetically almost identical
- Excellent candidates for determining additional markers for virulence

Objectives

- Sequence entire genome
- Identify differences
- Determine significance of differences using reverse genetics


Grateful for the assistance of
Mike Collins
for the ICPI work Bill Cox
Acknowledgment to groups who have submitted viruses to the EU reference laboratory



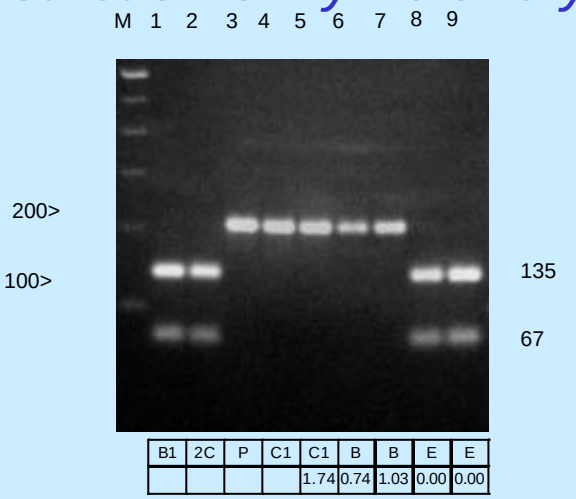
Diagnostic Protocol

- Screen by immunofluorescence (FITC-labelled polyclonal antiserum [PMV-1])
- Virus isolation in embryonated SPF eggs (92/66/EEC)
- ICPI determination
- Molecular diagnostics

Molecular diagnostics

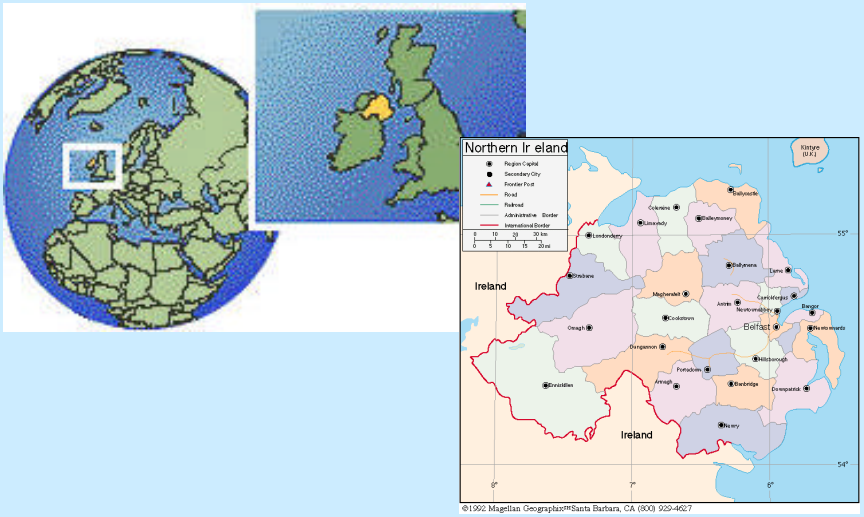
- One step RT-PCR -202bp, fusion protein cleavage site
- Restriction enzyme analysis- *Bgl*1.
  - Creelan, J.L., Graham, D.A. and McCullough, S.J. (2002) Detection of APMV-1 from field cases and differentiation of pathogenicity using one-step RT-PCR. Avian Pathology 31, 493-499.
- Sequencing

Restriction enzyme analysis



B1	2C	P	C1	C1	B	B	E	E
				1.74	0.74	1.03	0.00	0.00

Geography



Case 1

- 20/48 birds died. Adults vaccinated, juveniles not.
- First appeared in adults. Mainly juveniles died.

Date	Location	Sample	Age	HI/MAB	RT-PCR	Cleavage	Sequence	ICPI
22/6/4	C'fergus	Tissues	Adult	PPMV-1	Pos	Neg	^{112}R**RQKRF**^{117}	0.33

- Article 19 of 92/66/EEC applied-
  - -restriction on movement
  - -destruction/treatment of waste
- Vaccination 30d post recovery
- Press release to alert industry

Interpretation & ND definition

- 92/66/EEC: "... an infection of poultry caused by any avian strain of the paramyxovirus 1 with an ICPI in day-old chicks greater than 0.7.
- OIE: *An infection of birds caused by ...APMV-1 that meets one of the following criteria for virulence:*
  a) ICPI in day-old chicks of 0.7 or greater.
  or
  b) Multiple basic amino acids….. at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein.

Case 2

Loft visited by sick racing birds.
Also two stray birds from Scotland in loft preceding outbreak.
Trainer for other lofts. 15/80 died, mainly unvaccinated juveniles.

Date	Location	Sample	Age	HI/MAB	RT-PCR	Cleavage	Sequence	ICPI
22/6/4	C'fergus	Tissues	Adult	PPMV-1	Pos	Neg	^{112}R**RQKRF**^{117}	0.33

Case 2

24/6/4	C,fergus	Tissues	Juv	PPMV-1	Pos	Neg	^{112}R**RQKRF**^{117}	0.43
28/6/4	C,fergus	Swabs	-	PPMV-1	ND	ND	ND	ND

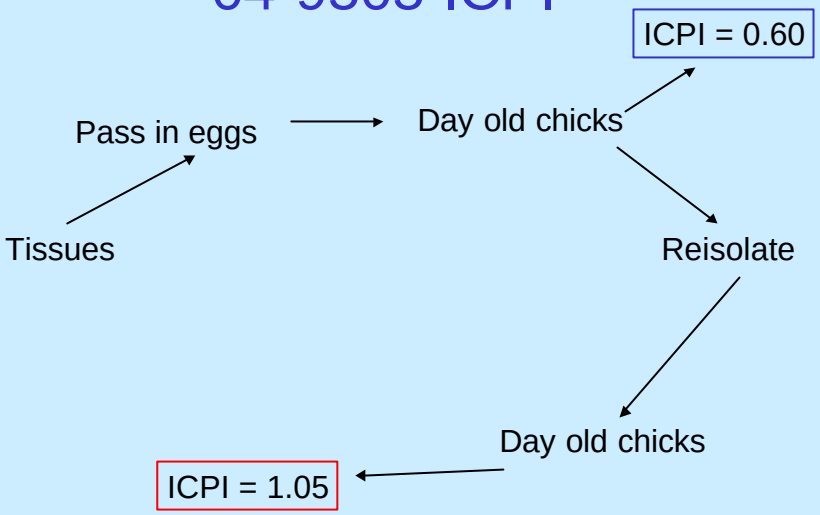
Case 3 History

- Belfast
- Two lofts , containing adults and young birds.
- Part of a larger collection of 30.
- All vaccinated with Colombovac.
- Only young birds sick, one death.

Case 3

Date	Location	Sample	Age	HI/MAB	RT-PCR	Cleavage	Sequence	ICPI
22/6/4	C'fergus	Tissues	Adult	PPMV-1	Pos	Neg	^{112}R**RQKRF**^{117}	0.33
24/6/4	C,fergus	Tissues	Juv	PPMV-1	Pos	Neg	^{112}R**RQKRF**^{117}	0.43
28/6/4	C,fergus	Swabs	-	PPMV-1	ND	ND	ND	ND
Case 3								
7/7/4	Belfast	Tissues	Juv	PPMV-1	Pos	Neg	^{112}R**RQKRF**^{117}	0.6
09/7/4	Belfast	Swabs	Juv	PPMV-1	Pos	Neg	^{112}R**RQKRF**^{117}	ND

04-9505 ICPI



Discussion/conclusions

- PPMV-1 isolates
  - ICPI typically greater than 1.0
    - Alexander et al. (1985), Av. Pathol. 14, 365-376.
  - ICPI <0.7 occasionally reported
    - Meulemans et al. (1986) Arch Virol. 87, 151-161.
    - Collins et al. (1994) Arch Virol. 134, 403-411.
  - Host adaptation.

Discussion/conclusions

- Molecular basis of virulence still incompletely understood
- Possible emergence of new strain
- Excluded from current EU definition and therefore pose potential risk through uncontrolled spread
- Definition should be expanded to include sequence data (actual or derived)
  - welfare, speed

Acknowledgements

- Staff of:
- Disease Surveillance and Investigation Branch
- Immunodiagnostic Branch
- Veterinary Service

NON EU OR USA ND ISOLATES and other issues

Ruth Manvell
CRL, VLA Weybridge

CYPRUS

- 3 isolates from pigeons
- ICPI = 1.80
- Sequence of RRQKRF

Saudi Arabia

- Several submissions from different areas throughout 2002 and 2004.
- Initial thoughts were that influenza H9 was responsible.
- Egg production problems then high mortality in multiple vaccinated birds.
- No inhibition with mAbs U85, 617/161 or 7D4.

Israel

- PPMV-1 isolates, ICPI = 1.24
- Vaccine strains (group E)
- 2 isolates of mAb goup C1, ICPI = 1.49

UAE

- Continuing problems with PPMV-1 infection of both pigeons and occasionally falcons.
- Virulent strains recently isolated similar to those isolated in Saudi Arabia and mAb group C1.
- Vaccination used routinely in many species.

Kosovo

- Virulent virus isolated from poultry.
- Sequence – RRQKRF
- ICPI – 1.70

Jordan

- Problems with ND - isolates similar to Saudi isolates.
- Vaccine strains

India

- Both vaccine and virulent strains were isolated. Virulent virus sequence RRQKRF

3 and 6 day passage of ND & AI viruses

- Dilutions of NDV Ulster and a H11 were inoculated into embryonated fowls eggs.
- One egg chilled at 3dpi was tested for HA activity, remaining eggs left until 6dpi and HA tested.
- Both 3 day and 6 day eggs were further passaged if HA negative

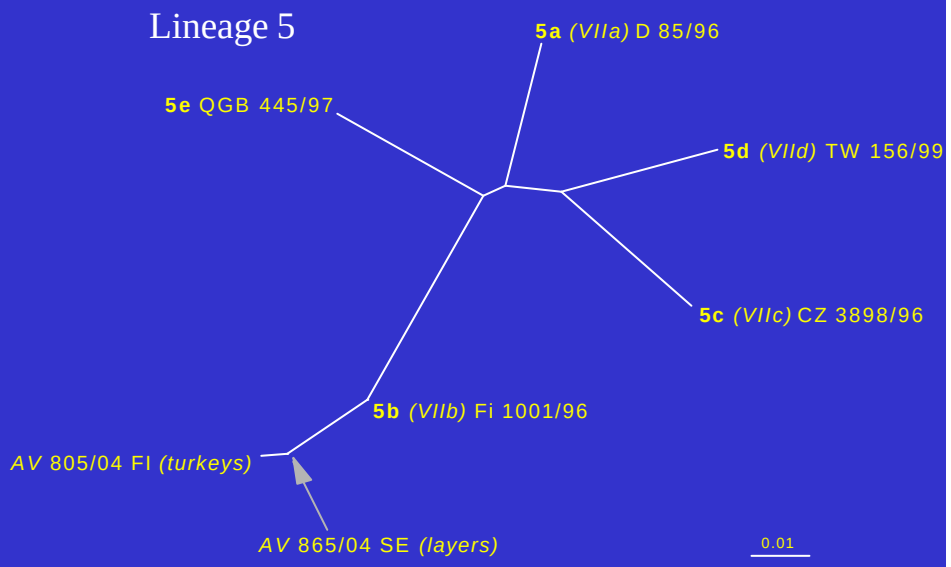
2 and 6 day passage of NDV

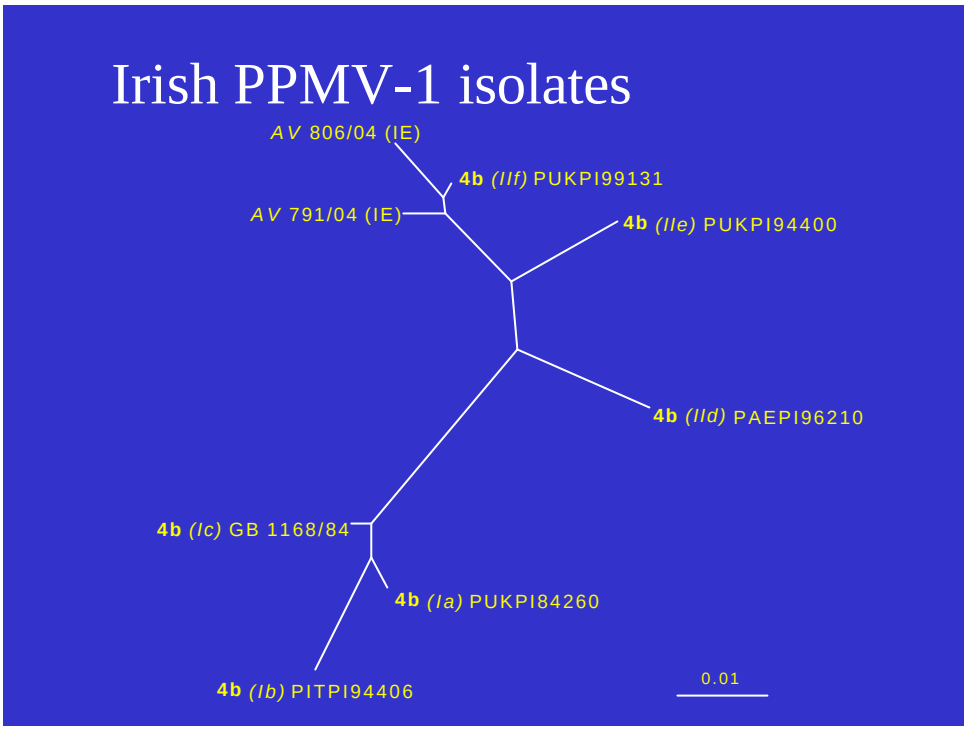
- Dilutions of –3, -6, -8 & -10 were a HA positive after 3days incubation. –12 –ve HA.
- -12 3dpi eggs were rapid passaged and tested after a further 3 days. All HA –ve.
- 2 x 6 day passages of –12 dilution also gave –ve HA result.

3 and 6 day passage of Flu

- -3 & -6 dilutions +ve HA after 3 dpi.
- -8, -10 & -12 dilution HA –ve after 2 x 3 day passage and also after 2 x 6 day passage.
- CONCLUSION:- No difference between 2 x 3dpi or 2 x 6dpi.

SCANDINAVIAN ISOLATES




Irish PPMV-1 isolates
AV 806/04 (IE)
4b (IIf) PUKPI99131
AV 791/04 (IE)
4b (IIe) PUKPI94400
4b (IId) PAEPI96210
4b (Ic) GB 1168/84
4b (Ia) PUKPI84260
4b (Ib) PITPI94406
0.01

COMPARATIVE TESTS FOR ANTIGEN IDENTIFICATION IN DIFFERENT NATIONAL LABORATORIES 2004

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INTRODUCTION

One of the functions and duties of the Community Reference Laboratories for Newcastle Disease and Avian Influenza is to organise "periodical comparative tests in diagnostic procedures at Community level". To fulfil this duty a simple test of the reproducibility in the National Laboratories of the haemagglutination inhibition [HI] test for the detection of Newcastle disease antibodies was organised in 1995 and for H5 and H7 influenza virus antibodies in 1997. While tests of the ability of the National Laboratories to identify Newcastle disease and influenza virus antigens were organised in 1998, 1999, 2001, 2002 and 2003. At the 9th Annual Joint Meeting it was felt that the antigen identification comparative tests were still revealing sufficient incorrect results to repeat the exercise and it was decided to send out 5 antigens for identification.

The objectives were to:

1. To test the ability of National Laboratories to determine the presence of notifiable disease.
2. To test the ability of National Laboratories not to confuse other viruses as notifiable.
3. To identify areas where improvements can be made.

As in the past, and following further consultation at the 9th Annual Meeting, results have been kept confidential to the submitting laboratory.

MATERIALS AND METHODS

Each National Laboratory was sent 5 unknown antigens with instructions to carry out identification of the antigens A-E by HA and HI tests. Laboratories were also asked to report the initial HA titre they had obtained with anitgens.

The antigens supplied were formalin or betapropiolactone inactivated whole viruses. Laboratories are expected to be at least able to identify H5 and H7 influenza viruses and APMV-1 [Newcastle disease] virus. However, implicit in this expectancy is that they will not erroneously identify other viruses as these. The antigens supplied were therefore selected to test these points. It was not

necessarily expected that every National Laboratory would fully identify all the antigens, but should be able to reach the minimum acceptable standard.

The antigens supplied and the minimum essential results were:-

Antigen	Virus	Minimum essential result
A	APMV-1 chicken/Ulster/2C/67	APMV-1
B	A/chick/Scotland/59 (H5N1)	H5
C	A/African starling/Eng-Q/983/79 (H7N1)	H7
D	A/turkey/Ontario/7732/66 (H5N9)	H5
E	A/chick/Scotland/59 (H5N1)	H5

RESULTS

Antigen identification

Twenty-seven laboratories of the 31 that had been sent samples responded by submitting results. Samples should have been received by the laboratories by 2nd September 2004 and the deadline for returning results was 25th September 2005. The results are shown in Table 1. Twenty-four EU laboratories responded, this included an additional laboratory for N. Ireland and separate influenza and Newcastle disease laboratories for Greece. Belgium acts as both reference laboratories for Luxembourg. Laboratories from 3 non-EU states participated these were: Bulgaria, Romania and Switzerland.

In total 134 results were received from the 27 laboratories. The correct results were obtained on 126 [94.0%] occasions. Eight [6.0%] were wrong. Two laboratories, 8 and 12, identified antigen 1 as PPMV-1, which was considered an incorrect result even though APMV-1 would have been correct. Antigen 1 was Ulster 2C, which is the recommended reference strain and as such should have been easily identifiable as APMV-1, not PPMV-1. One laboratory, 3, considered antigens 2 and 5 were inhibited by both H5 and H7 antisera and this was considered a wrong result – although at least the laboratory was consistent as these were identical antigens. All other laboratories correctly identified these as H5. All laboratories correctly identified antigen 3 as H7 [the second year running all laboratories had been able to identify the H7 antigen correctly]. However, the antigen 4 did cause some problems with 4 incorrect results ranging from PMV-2 [laboratory9], PNV-3 [22] H6 [7] and 'not H5 or H7' [23]. Some laboratories volunteered neuraminidase subtype identification, all were correct except laboratory 2 identified antigen 5 as N3 instead of N1 and laboratory 9 identified the same antigen as N7. Strangely both these laboratories identified antigen 2 correctly as N1,

Of the 27 participating laboratories, 20 fully identified all HA. Six laboratories had one unacceptable result and one had two unacceptable result.

Haemagglutinin titres

This year the laboratories were asked to supply the HA titres obtained for the antigens supplied that were used to calculate the 4 HAU. Only 12 of the laboratories supplied their results (Table 2). Generally there was and 8-fold difference for each antigen between the highest and lowest titres from the

different laboratories. There was some consistency in that laboratories tended to show higher or lower results than the median titre for each antigen.

DISCUSSION

One of the objectives of the comparative tests is that laboratories should be able to take remedial measures where they have fallen short of the desired standard. The results obtained for 2004 compared to those in recent years indicate that such improvements are being made for overall results:

	2000	2002	2003	2004
Wrong	8.7%	13.0%	11.0%	6.0%
Not wholly correct	9.8%	0.7%	2.0%	0%
Correct	81.5%	86.0%	87.0%	94.0%

All 27 laboratories taking part in 2004 all had taken part in 2003. The comparative results for the two years were:

Number that:-

	2003	2004
Satisfactorily identified all antigens:	18	20
Had one unacceptable result	6	6
Had more than one wrong	3	1

In fact compared to the 2003 test performance 4 laboratories showed an improvement; 16 were the same with all results correct; two were the same with one incorrect result and 5 laboratories obtained worse results than 2003. No country fell into any other possible category.

The antigen HA titration results were disappointing, both in the response and in the results obtained. For virus identification it is less important but the results suggest that in HI tests some laboratories may be using up to 8 times more or 8 times less antigen than other laboratories use. Possibly even more disappointing is that both Directive EEC/92/66 and the OIE Manual recommend that the HA titre is calculated initially from a close series of dilutions i.e. 1/3, 1/4, 1/5, 1/6 etc. From the results presented 10 of the 12 laboratories *and the CRL* would seem to have titrated in doubling dilutions directly from the reconstituted antigen. This may not greatly affect the titres obtained [compare other titres with laboratory 24], but laboratories may find that this is considered as failure to comply with standard practices when they are assessed for quality accreditation.

Table 1. Results of comparative antigen identification tests

LABS	**ANTIGENS**				
	1	2	3	4	5
CRL	***APMV-1***	***H5N1***	***H7N1***	***H5N9***	***H5N1***
1	APMV-1	H5N1	H7N1	H5N9	H5**N3**
2	APMV-1	H5	H7	H5	H5
3	APMV-1	H5 **& H7**	H7	H5	H5 **& H7**
4	APMV-1	H5N1	H7	H5N9	H5
5	APMV-1	H5	H7		H5
6	NDV	H5	H7	H5	H5
7	APMV-1	H5	H7	**H6**	H5
8	**PPMV-1?**	H5	H7	H5	H5
9	APMV-1	H5 (N1?)	H7 (N1?)	PMV-2	H5 **(N7?)**
10	APMV-1	H5	H7	H5	H5
11	NDV	H5	H7	H5	H5
12	**PPMV-1?**	H5	H7	H5	H5
13	APMV-1	H5	H7	H5	H5
14	APMV-1	H5	H7	H5	H5
15	APMV-1	H5N1	H7N1	H5	H5N1
16	APMV-1	H5N1	H7N1	H5N9	H5N1
17	APMV-1	H5	H7	H5	H5
18	APMV-1	H5	H7	H5N9	H5
19	NDV	H5	H7	H5	H5
20	APMV-1	H5	H7	H5	H5
21	APMV-1	H5	H7	H5	H5
22	APMV-1	H5	H7	PMV-3	H5
23	APMV-1	H5	H7	**Not H5 or H7**	H5
24	APMV-1	H5N1	H7N1	H5	H5N1
25	APMV-1	H5	H7	H5	H5
26	APMV-1	H5 (N1)	H7 (N1)	H5	H5
27	APMV-1	H5 (N1)	H7 (N1)	H5	H5

Table 2. Results of comparative antigen haemagglutinin titres

LABS	ANTIGENS				
	1	**2**	**3**	**4**	**5**
CRL	128	128	64	64	128
1	64	64	64	32	64
2	128	128	32/64	16/32	128
3	64	128	64	128	128
10	64	32	8	16/32	32
11	256	128	64	64	128
13	64	256	64	64	128
15	32	64	32	32	64
16	256	128	64	32	128
20	64	128	32	16	128
21	19	38	19	33	55
24	190	134	67	47	134
25	256	256	64	64	256

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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E2 - Animal health and welfare, zootechnics

SANCO/10544/2004
Working document

COMMUNITY REFERENCE LABORATORIES FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE

WORK PROGRAMMES 2005

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2005

I. LEGAL FUNCTIONS AND DUTIES

The functions and duties are specified in Annex V of Council Directive 92/40/EEC (Official Journal of the Communities No L 167 of 22.6.1992).

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2005

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:
   a) Determining the intravenous pathogenicity index (IVPI)
   b) Antigenic typing of viruses and both haemagglutinin and neuraminidase subtypes
   c) Determining the amino acid sequence at the haemagglutinin cleavage site of H5 and H7 subtype viruses
   d) Limited phylogenetic analysis to assist in epidemiological investigations.
2. Maintain and distribute virus repository and reagents necessary for virus characterisation.
3. Prepare and distribute antisera, antigens and reagents for the inter-laboratory comparison tests.
4. Analysis of results submitted by National Laboratories for the inter-laboratory comparison tests.
5. Conduct work to evaluate reported problem areas in diagnosis.
6. Supporting by means of information and technical advice National Avian Influenza Laboratories and the European Commission during epidemics.
7. Prepare the programme and working documents for the Annual Meeting of National Avian Influenza Laboratories.
8. Collecting and editing of material for a report covering the annual meeting of National Avian Influenza Laboratories.
9. Carry out work in relation to the surveys for avian influenza in poultry and wild birds implemented by Member States during 2004/05, revision of guidelines and production of final report.
10. In the light of the occurrence of influenza in birds and other animals keep under review the possible zoonotic impact arising from the risk of reassortment between influenza viruses.
11. Preparation and publications of articles and reports associated with above work.

It is understood that the above mentioned objectives are not exclusive to other work of more immediate priority which may arise during the given period.

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2005

I. LEGAL FUNCTIONS AND DUTIES

The functions and duties are specified in Annex V of Council Directive 92/66/EEC (Official Journal of the European Communities No L 260 of 5.9.1992).

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2005

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:
   a) Determining the intracerebral pathogenicity index (ICPI)
   b) Determining basic amino acids composition adjacent to the cleavage site of the FO protein in the virus and phylogenetic analysis
   c) Antigenic grouping of viruses
   d) Limited phylogenetic analysis to assist in epidemiological investigations.
2. Maintain and distribute virus repository and reagents necessary for virus characterisation.
3. Prepare and distribute antisera, antigens and reagents for the inter-laboratory comparison tests.
4. Analysis of results submitted by National Laboratories for the inter-laboratory comparison tests.
5. Conduct work to evaluate reported problem areas in diagnosis.
6. Supporting by means of information and technical advice National Newcastle Disease Laboratories and the European Commission during epidemics.
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It is understood that the above mentioned objectives are not exclusive to other work of more immediate priority which may arise during the given period.

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