EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate E - Food Safety: plant health, animal health and welfare, international questions
E2 - Animal health and welfare, zootechnics

SANCO/10436/2004

Joint 9TH Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of EU Member States

BRUSSELS (BELGIUM), 11 – 12 DECEMBER 2003

Proceedings

(Edited by Dennis J. Alexander)

European Commission, B-1049 Brussels-Belgium

CONTENTS

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DENMARK	Dr Jorgen Westergaard

JOINT NINTH ANNUAL MEETINGS OF THE NATIONAL NEWCASTLE DISEASE AND AVIAN INFLUENZA LABORATORIES OF COUNTRIES OF THE EUROPEAN UNION, 2003 HELD IN BRUSSELS, BELGIUM

PROGRAMME FOR THURSDAY 11 DECEMBER 2003

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

9:15 − 9:30	Welcome	
9:30 − 10:00	Report from the EU Reference Laboratory	*D. Alexander*
10:00 − 10:45.	Avian influenza in the Netherlands	*G. Koch*
10:45 − 11:15	Current AI situation in Italy	*I. Capua*
11:15 − 11:45	*Coffee*	
11:45 − 12:45	Original contributions on AI	
11:45 – 12:00	AI test validation	*G Koch*
12:00 – 12:15	A competition NP-based AI ELISA for antibodies in ducks	*V. Jestin*
12:15 – 12:30	Three AI virus detection methods compared to virus isolation	*G.Cattoli*
12:30 – 12:45	Lab organisation/sample processing during the Dutch HPAI	*G.Koch*
12:45 − 14:00	*Lunch*	
14:00 − 14:30	Original contributions on AI continued	
14:00 – 14:15	Susceptibility/shedding of vaccinated turkeys infected with LPAI.	*C.Terregino*
14:15 – 14:30	LPAI compared with HPAI in meat	*D. Swayne*
14:30 – 15:00	Survey for AI in poultry and wild birds	*I. Brown*
15:00 − 15:30	Simulation exercises for AI/ND	*J. Westergaard*
15:30 − 16.00	*Coffee*	
16.00 − 16:30	Country reports on AI based on questionnaires	*D. Alexander*
16:30 − 17.00	Proposed USDA H5 and H7 control plan	*D. Swayne*
17:00 − 17:30	Discussion	

PROGRAMME FOR FRIDAY 12 DECEMBER 2003

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

9:15 − 9:45	Country reports on ND based on questionnaires	*D. Alexander*
9:45 − 10:15	Report from the European Commission	*M. Pittman*
10:15 − 11:00	Newcastle disease in the USA	*D. Senne*
11:00 − 11:30	*Coffee*	
11:30 – 12:00	ND in Denmark	*S. Mortensen*
12:00 – 13:00	Original contributions on ND	
12:00 − 12:15	Egg Pasteurisation and inactivation of ND and AI	*D. Swayne*
12:15 − 12:30	Immunity and transmission of ND	*G. Koch*
12:30 – 12:45	Molecular epidemiology of PPMV-1 viruses	*I. Brown*
12:45 – 13:00	Effect of F0 cleavage site mutation on NDV virulence	*G. Koch*
13:00 − 14:00	*Lunch*	
14:00 − 14:30	Interlaboratory comparative tests	*D. Alexander*
14:30 − 15:00	ND situation worldwide excluding EU and USA	*R. Manvell*
15:00 − 15:15	Work plan of the Community Reference Laboratory for 2004	
15:15 − 15:30	Discussion and close	

Annual Meeting of the National Laboratories for Avian Influenza

TECHNICAL REPORT OF THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2002

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 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 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**
401	188	113	154	199	294	385

1995	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**
605	284	227	285	357	704	316	333

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: *59 %*

WORK DONE: The viruses submitted in 2002 were characterised as shown in Table 2.

Table 2: Identification of viruses submitted to the CRL in 2002

Virus identification	Number
Paramyxoviruses	**178**
Influenza A viruses	**137**
H1N2	2
H3N2	1
H3N8	4
H4N6	1
H6N1	1
H6N2	5
H6N8	1
H7N3	63
H7N7	2
H9N2	55
H11N3	1
H11N9	1
others	***18***
reovirus	2
poxvirus	2
untyped	2
virus not viable	12

In addition to conventional typing of the viruses submitted a total of 15 representative H7 viruses was subjected to nucleotide sequencing and the amino acids at the haemagglutinin cleavage site deduced.

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

Estimated actual resources: 62%

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*: 7 %*

WORK DONE: The AI viruses received were added to the repository. Reagent stocks were maintained, at least at previous levels [Table 3] although the demand for reagents was much higher than usual and during the year the following were supplied:

ANTIGENS - 4 x 1.0ml ampoules of influenza A agar gel precipitin antigen, 5 x 1.0ml of H1N1, 100 x 1.0ml of H5N1, 126 x 1.0ml of H5N2, 163 x 1.0ml of H5N9, 90 x 1.0ml of H7N1, 90 x 1.0ml of H7N7 and 212 x 1.0ml of H9N2; plus 9 x 1.0ml of Equine/Miami/1/63, 9 x 1.0ml of Equine/Prague/1/56 and 9 x 1.0ml of Equine/Fontainbleu/79

ANTISERA - 7 x 0.5ml of H1N1, 2 x 0.5ml of H1N2, 2 x 0.5ml of H2N2, 2 x 0.5ml of H2N3, 4 x 0.5ml of H3N2, 2 x 0.5ml of H4N6, 34 x 0.5ml H5N1, 39 x 0.5ml H5N2, 10 x 0.5ml H5N9, 4 x 0.5ml H6N6, 28 x 0.5ml H6N8, 44 x 0.5ml H7N1, 4 x 0.5ml H7N3, 12 x 0.5ml H7N7, 2 x 0.5ml H8N4, 12 x 0.5ml H9N2, 4 x 0.5ml H9N7 and 28 x 0.5ml of influenza A agar gel precipitin antiserum.

Also 15 x 0.5ml SPF chicken serum was 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	150	8	300	7
H7	200	7	400	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 and antisera were prepared and dispatched to EU National Laboratories and those of accession countries [total 29 laboratories]

Estimated actual % resources**:* ***6%

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 2002. 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 and the host's representatives the Annual Meeting was organised and held in Padova Italy in June 2002.

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 delayed 2001 proceedings before 2002 Annual meeting. Receive and collate submissions of 2002 meeting.*

***% Resources**: 2 %*

WORK DONE: Proceedings of the 2001 meeting were produced, the Proceedings of the 2002 meeting are being edited, some submissions had not been received by the end of 2002.

Estimated actual % resources**: **2%

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: Due to delays no surveillance results were available for analysis.

Estimated actual % resources**: **0%

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 SCFCAH and reviewing proposed national surveillance programmes.*

***% Resources:** 8%*

WORK DONE: CRL staff attended 3 SCFCAH committee meetings, one meeting of the National Laboratories and critically reviewed all proposed surveillance programmes for member states.

Estimated actual % resources**: **8%

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

***% Resources:** 1%*

WORK DONE:

RELEVANT PUBLICATIONS IN 2002

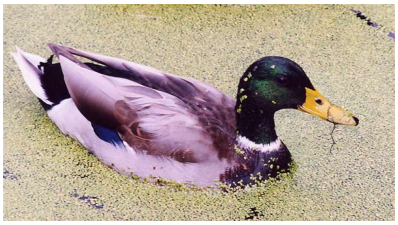
1. STANISLAWEK, W.L., WILKS, C.R., MEERS,J., HORNER, G.W., ALEXANDER, D.J., MANVELL, R.J, KATTENBELT, J.A. & GOULD, A.R. (2002). Avian paramyxoviruses and influenza viruses isolated from mallard ducks (*Anas platyrhyncos*) in New Zealand. Archives of Virology 147, 1287-1302.
2. CAPUA, I &. ALEXANDER, D.J. (2002). Avian influenza and human health. Acta tropica 83, 1-6.
3. ALEXANDER, D.J. & MANVELL, R.J. (2002). Report of the European Union Reference Laboratories for avian influenza and Newcastle disease 2000. Proceedings of the Joint 7th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Uppsala, April 2001 pp 6-13
4. ALEXANDER, D.J. & MANVELL, R.J. (2002). Country reports on avian influenza and Newcastle disease for 2000 based on responses to the questionnaire. Proceedings of the Joint 7th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of countries of the European Union, Uppsala, April 2001 pp 22-49
4. ALEXANDER, D.J. & MANVELL, R.J. (2002). Comparative tests for antigen identification in different National Laboratories 1999. Proceedings of the Joint 7th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Uppsala, April 2001 pp 50-55
5. ALEXANDER, D.J. (2002). Report on avian influenza in the Eastern Hemisphere during 1997-2002. Abstracts of the 5th International Symposium on Avian Influenza, Athens, Georgia, April 14-17 2002. pp 9-10.
6. ALEXANDER, D.J. (2002). Should we change the definition of avian influenza for eradication purposes? Abstracts of the 5th International Symposium on Avian Influenza, Athens, Georgia, April 14-17 2002. p 22.
7. ALEXANDER, D.J. (2002). Influenza. Abstracts of The Prevention and Control of Zoonoses International Conference October 2002 Cardiff. p 13.
8. BROWN, I.H., ESSEN, S.C, HARRIS, P.A, MYNN, J. & ALEXANDER, D.J. (2002). Genetic diversity in the internal protein genes of influenza A viruses from recent Eurasian avian and swine viruses. Abstract p-W6-5 The 1st European influenza conference, Malta 2002, ESWI p 76
9. BANKS, J., AHERNE, R.J., BROWN, I.H., ESSEN, S.C, & ALEXANDER, D.J. (2002). Genetic diversity in the internal genes of H9 influenza A viruses. Abstract p-W6-7 The 1st European influenza conference, Malta 2002, ESWI p 76
10. MANVELL, R.J., (2002) Abstracts of European Branch of the Poultry Veterinary Services Group Meeting 8th November 2002 Edinburgh, U.K. Laboratory Investigations into Influenza in Chile.
11. MANVELL, R.J., ENGLISH, C., JORGENSEN, P.H., BROWN, I.H. (2002) Pathogenesis of H7 Influenza Viruses Isolated from Ostriches in the Homologous Host Infected Experimentally. Abstracts of The 5th International Symposium on Avian Influenza, Athens, Georgia, USA April 14-17.

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

CIDC-LELYSTAD WAGENINGEN UR

AI IN WILD WATERFOWL IN THE NETHERLANDS

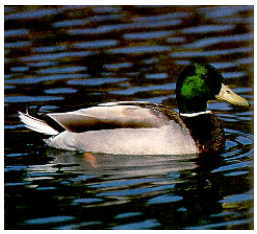
- In 1999 subtype H10N7 and 2000 subtype H7N3 was isolated from mallard (*Anas platyrhynchos*) in the Netherlands



CIDC-LELYSTAD WAGENINGEN UR

AI OUTBREAK IN THE NETHERLANDS

- Subtype H7N7 of the outbreak 2003 is probably a reassortment of both viruses and circulated as a LPAI virus in waterfowl



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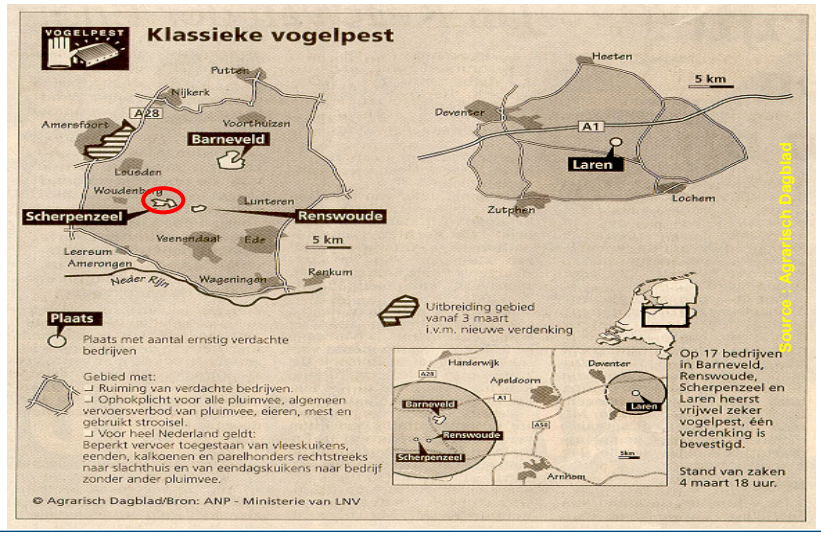
POSSIBLE ROUTE OF ENTRY OF AI



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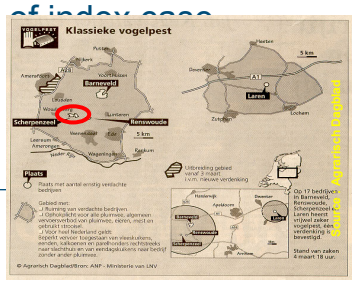
POSSIBLE ROUTE OF ENTRY OF AI VIRUS

- Free range laying hens infected by LP H7N7 variant of wild fowl
- Circulated in one stable of index case indicated by positive serology in absence of signs in this stable
- Mutation and selection of HP-variant in chickens
- Clinical signs of AI in other stable of index case



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VIRUS CHARACTERISTICS

- A/chick/2003-H7N7
- All genes are of avian origin
- IVPI = 2,94
- Sequence at cleavage site:
  - P E I P K R R R R . G L F
- H7 closely related with A/mallard/2000 H7N3
- N7 closely related with A/mallard/1999 H10N7
- No additional glycosylation sites
  (suggest recent introduction from wild fowl)

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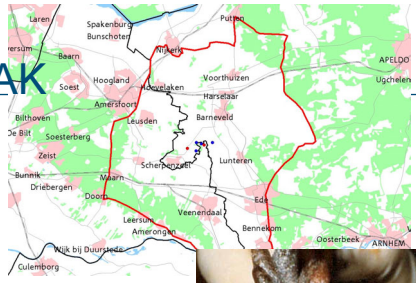
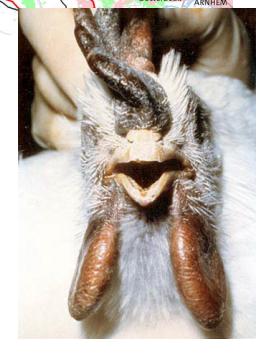
START OF AI OUTBREAK



<u>Friday February, 28 2003:</u>
Strong suspicion of AI on 6 layer farms in Gelderse Vallei



Signs:
Mortality (> 80%), drop in egg production, decreased food consumption, swollen heads, cyanosis, diarrhea and respiratory problems.

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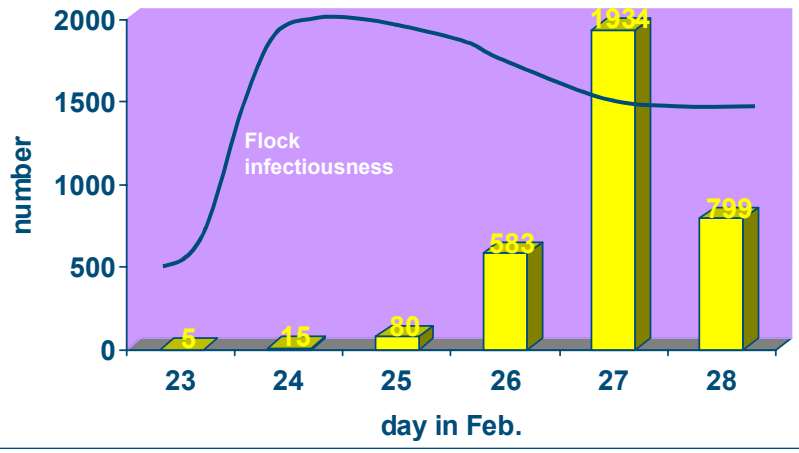
EXAMPLE MORTALITY

(flock of 3800)



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ROUTES OF TRANSMISSION

- Men
- Egg transport
- Feed
- Most infection routes are unknown
  - 40% spread within 1 km
  - 17% feed lorries
- Contaminated dust?
  - Forced ventilation.

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CONTROL MEASURES

Stand still

- Reduction of number contacts

(Pre-emptive) culling

- Reduction of infectiousness
  - Infected farms should be culled within 24 hours
- Reduction of number of susceptible birds (farms)
  - Pre-emptive culling within 48 hours

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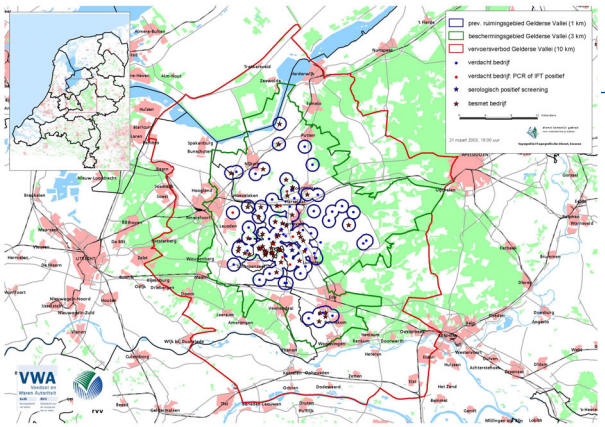
CONTROL OF AI (1)



March 1 : already stand-still ±20 farms infected
March 4 : infected farms culled
+ pre emptive culling within 1 km ± 46 herds infected

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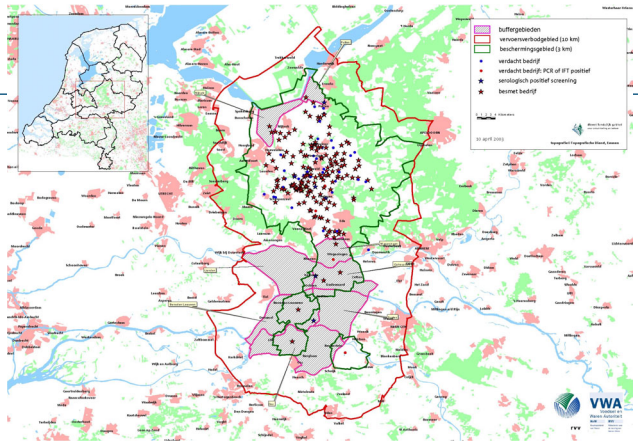
CONTROL OF AI (1)



March 25 : culling of buffer regions
(Wageningen en Putten)
March 25 : Beneden Leeuwen (March 30)

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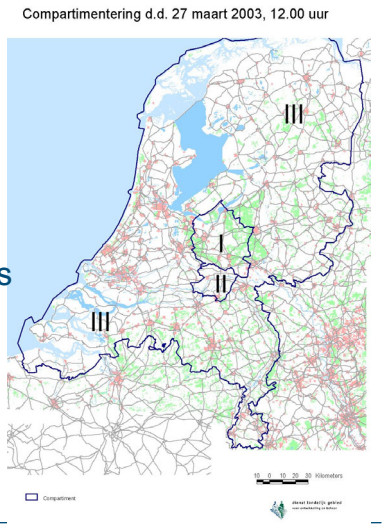
- March 27 : setting up of compartments



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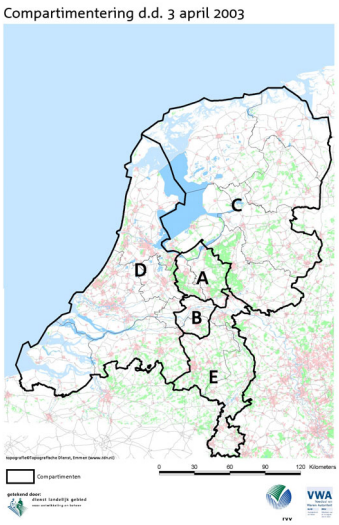
- March 27 : setting up of compartments
- April 1 : culling of all flocks in protection zone GV
- April 3 : Ospel (Nederweert) (7 April)



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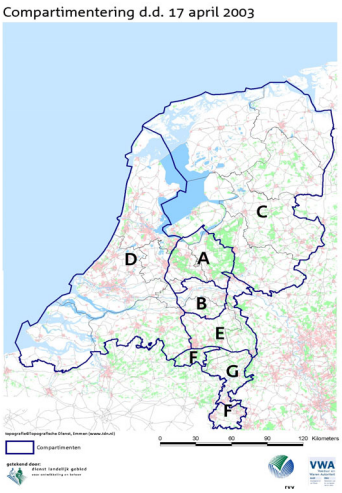
- March 27 : setting up of compartments
- April 1 : culling of all flocks in protection zone GV
- April 3 : Ospel (Nederweert) (7 April)
- April 4 : 2e Stand-still (till April 10)
- April 10 : Koningsbosch (April 14)



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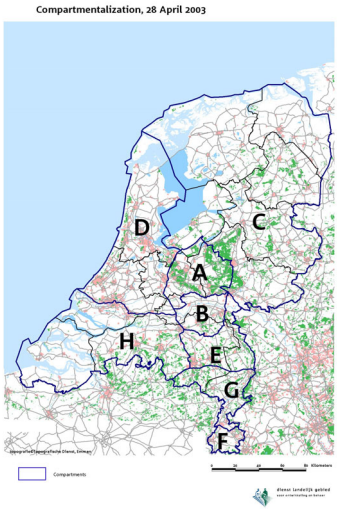
- March 27 : setting up of compartments
- April 1 : culling of all flocks in protection zone GV
- April 3 : Ospel (Nederweert) (7 April)
- April 4 : 2e Stand-still (till April 10)
- April 10 : Koningsbosch (April 14)
- May 5 : Wernhout (Zundert) (May 9)
- May 11 : last infected farm culled



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ANALYSIS OF DATA

- Model: $C_{\delta t} = \beta \times I \times S \times \delta t / N$
  - $C_{\delta t}$: number of outbreaks in period δt
  - β: infection rate parameter (average number of new infections per infectious flock per time period)
  - I: number of infectious flocks
  - S: number of susceptible flocks
  - N: total number of flocks

  Generalized Linear Model, using Poisson distribution and a log link function

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INFECTION RATE PARAMETER (β) IN THE GELDERSE VALLEI AND LIMBURG BEFORE AND AFTER THE IMPLEMENTATION OF MEASURES

	Gelderse Vallei		Limburg	
Period	**A**	**B+C**	**A**	**B+C**
β	**0.42 (0.27-2.41)**	**0.17 (0.11-0.28)**	**0.42 (0.17-3.65)**	**0.18 (0.08-0.46)**

95% confidence intervals between brackets

A, before implementation of measures, B and C, after implementation of measures. Periods B and C were combined, because of non-significant differences J.A. Stegeman et al 2003

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STOP AN EPIDEMIC BY STAMPING OUT

- Flock must be depopulated before it has infected on average more than one other flock
- R_h, reproduction ratio between flocks: average number number of infections caused by one infected flock

CIDC-LELYSTAD WAGENINGEN UR

TRANSMISSION BETWEEN FLOCKS

- **R_h** = average number of outbreaks caused by one infected herd

$$R_h = ß \times T$$

  - **ß** = average number of new infections caused by one infected flock per week
  - **T** = average infectious period

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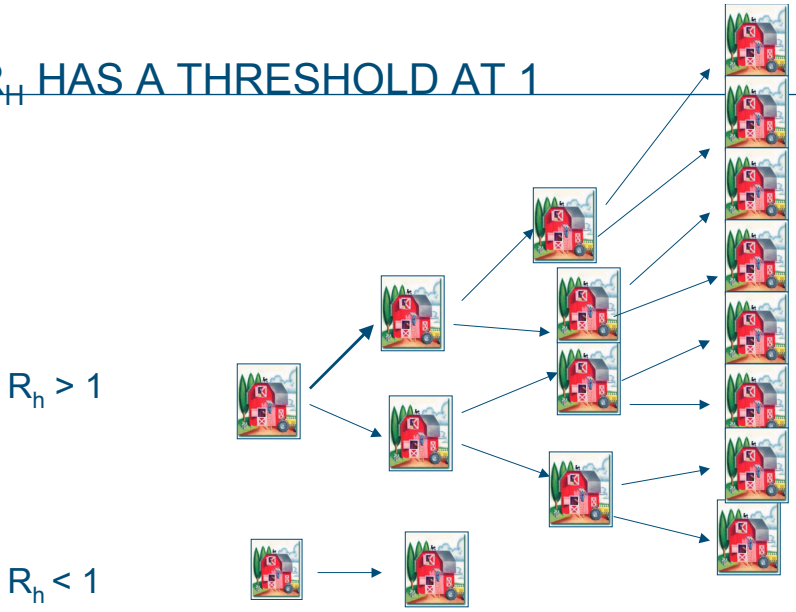
R_H HAS A THRESHOLD AT 1



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INFECTION RATE PARAMETER (β) AND REPRODUCTION RATIO R_H BEFORE AND AFTER THE IMPLEMENTATION OF MEASURES

	Gelderse Vallei		Limburg	
Period	**A**	**B+C**	**A**	**B+C**
β	**0.42 (0.27-2.41)**	**0.17 (0.11-0.28)**	**0.42 (0.17-3.65)**	**0.18 (0.08-0.46)**
R_h	**5.0 (2.9-8.6)**	**0.91 (0.39-2.13)**	**2.9 (no CI)**	**0.86 (0.28-2.68)**

95% confidence intervals between brackets

A, before implementation of measures, B and C, after implementation of measures. Periods B and C were combined, because of non-significant differences

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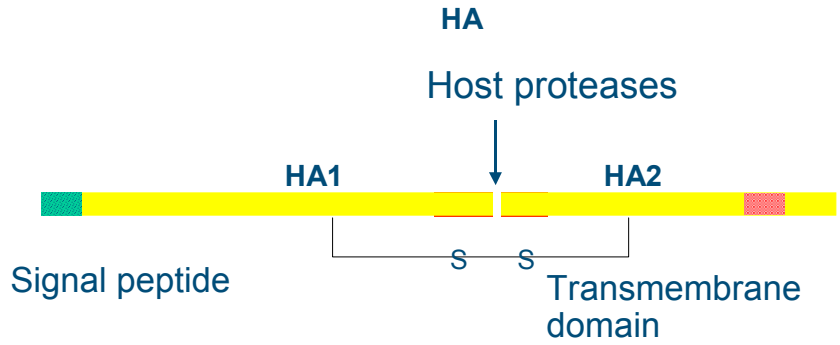
VIRULENCE FACTOR

Haemagglutinin (HA)
Mol. Weight: 75,000-80,000 Da



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Sampling

Suspected:

- 5 euthanised chickens
  - Trachea, lung, spleen
- 20 blood samples

Preventive culling within 1 km:

- 20 trachea swabs
- 20 blood samples

Preventive killing outside 1 km:

- 20 blood samples

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Tests used:

Agent detection

- Virus isolation in embryonated eggs (Annex II, 92/40).
- RT-PCR H7 and matrix gene.

Antibody detection

- Blocking DAS-ELISA (antibodies against nucleoprotein).
- Haemagglutination inhibition test using H7N7 as antigen.

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PCR DETECTION AVIAN INFLUENZA A VIRUSES

- Gene of matrix protein.
- This gene is strongly conserved since the gene comprises two overlapping reading frames.
- Detects all influenza A viruses.
- Detects all other influenza viruses and thus also control for the H7 RT-PCR.
- Farm is considered infected if both the H7 en M RT-PCR are positive.

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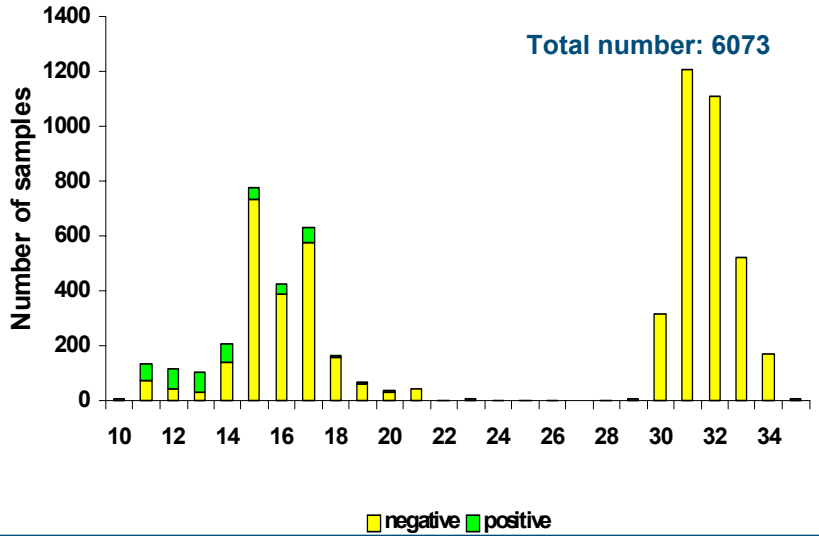
AVIAN INFLUENZA: RT-PCR



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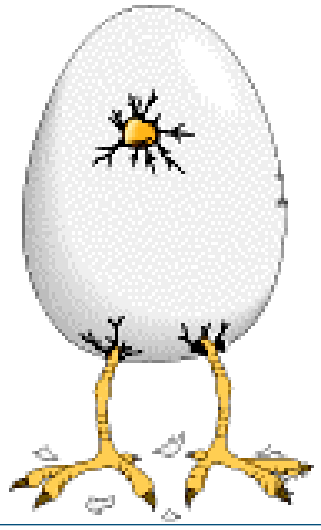
LABORATORY DIAGNOSIS OF AVIAN INFLUENZA

- Mean death time 48-72 hours.
- Haemagglutinin activity
- Typing using polyclonal antibody



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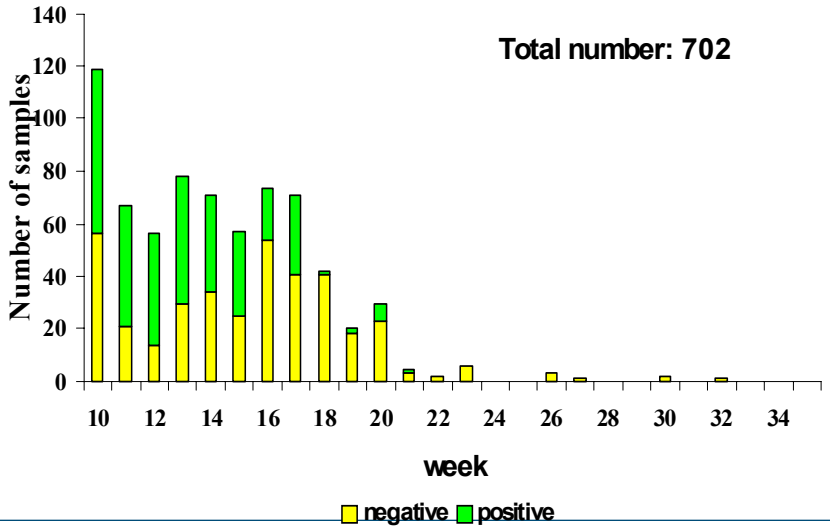
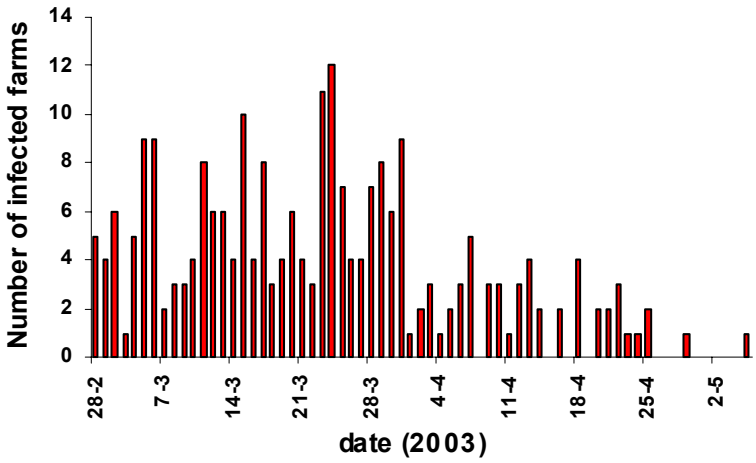
AVIAN INFLUENZA - VIRUS ISOLATION



CIDC-LELYSTAD WAGENINGEN UR

NUMBER INFECTED FARMS: 241



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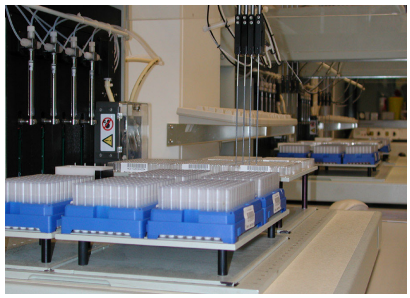
RAW RESULTS OF ANTIBODY DAS-ELISA



Total number	Number negative	Number positive	Number aspecific	Number tested in HAI
121835 100%	108716 89,2 %	6678 5,5%	6441 5,3%	13119 10,8%

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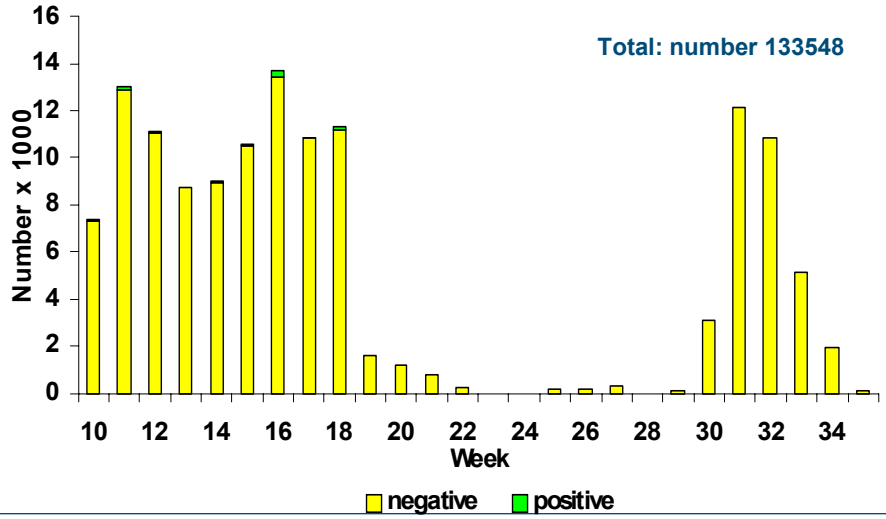
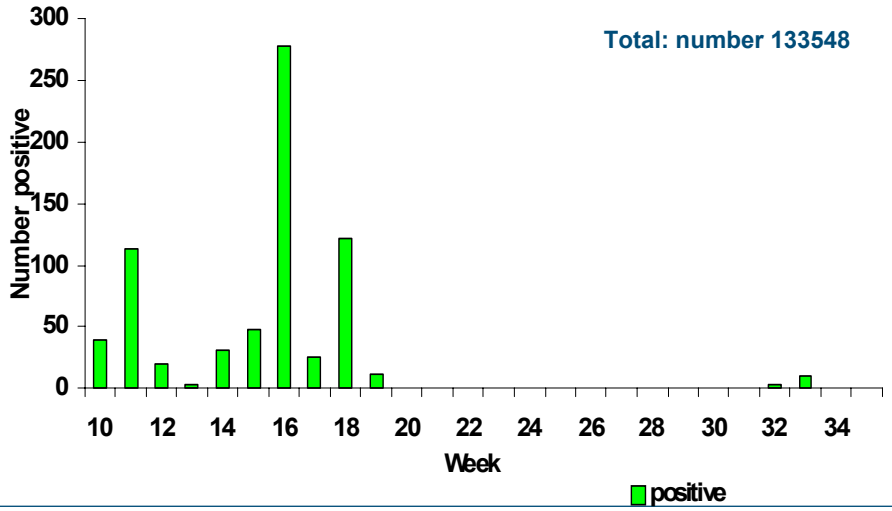
SEROLOGY IN INFECTED ZONES



CIDC-LELYSTAD WAGENINGEN UR

SEROLOGY IN INFECTED ZONES



CIDC-LELYSTAD WAGENINGEN UR

RESULTS SEROLOGAL AI-MONITORING IN THE INFECTED REGIONS

Status of farm	Virus isolation	Number of farms	Positive H_7	Positive H_5
Infected	+	241	5	0
	-	14	14	0
Pre-emptive	N.d.	1061	0	23¶

¶ Prevalence within farm varies form 9 –100 %

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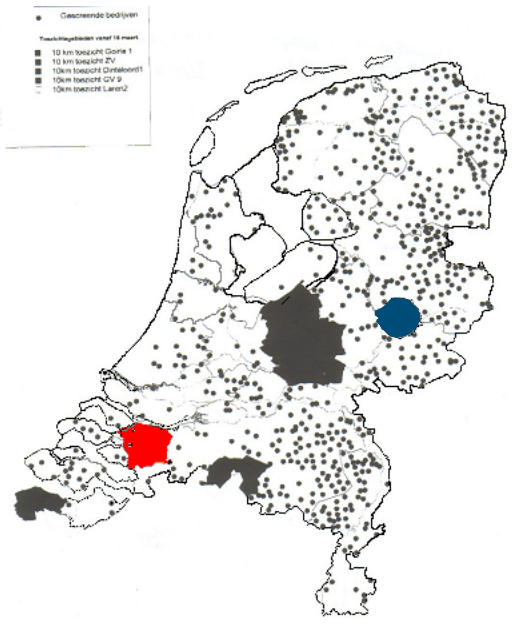
LOCATION OF FARMS IN THE LPAI MONITORING



CIDC-LELYSTAD WAGENINGEN UR

RESULTS OF SEROLOGY AI-MONITORING OUTSIDE INFECTED REGIONS IN WEEK 7

Category	Total number	Number sampled	Number positive H_7¶
Lay	849	309	0
Free range Lay	94	135	2
Broilers	1143	359	0
Breeders	540	212	0
Turkey	104	100	1
Duck/goose	100	78	0
Total	2830	1193	3

¶ Two other farms scored positive for other subtypes (H_1 (North Limburg) and ? (Drente)

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SCREENING:

- Ostriches with tracheitis
- Farm in Heeten (Raalte)
- A/Ostriche/Neth/2003/3006814-H2N3

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AVIAN INFLUENZA INTRODUCTIONS

December 2002-March 2003

- Minimal 6 separate introductions.
  - H7N3
  - H7N7
  - H5N?
  - H2N3
  - H1N1
  - Unknown subtype
- In three of these events free farms were involved.

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INFECTION OF H7N7 IN PIGS

- 48 farms with mixed herds which were located within the protection zones and had infected poultry were tested for antibodies.
- On 13 farms prevalence's between 2,2- 42% were detected.
- No increase in prevalence after retesting of 5 infected farms
  - 173-713 samples per herd, total 2373 samples analysed.
- 60 oropharyngeal swabs of these farms were all negative.
- No evidence of transmission among pigs.
- All pigs of positive farms were slaughter under official control.
- Non-specific reactivity of swine sera in H7 haemagglutination inhibition test.

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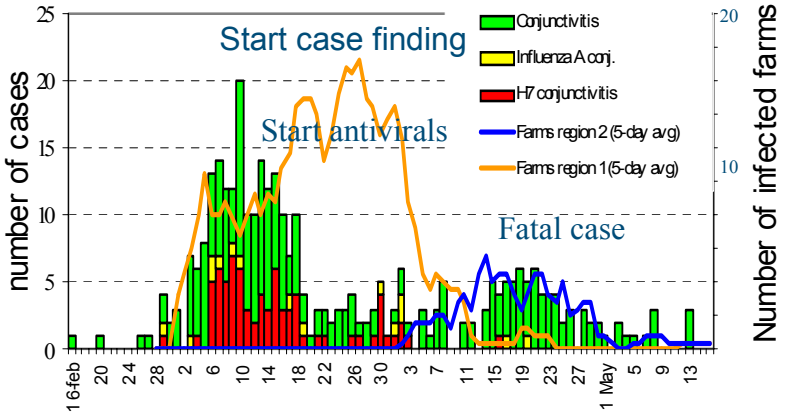
HUMAN CASES



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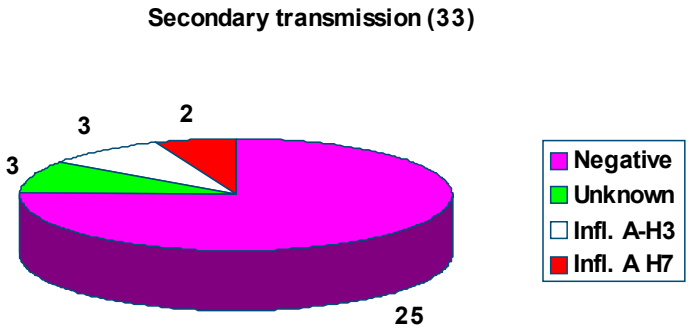
HUMAN CASES (3)



CIDC-LELYSTAD WAGENINGEN UR

- **Direct costs: 270 million**
- **Indirect costs: 1 milliard**
- **Other animals (pigs) are susceptible**
- **Consequences for public health**
- **Increased risk for new pandemic (reassortment)**
- **Damaged image of the poultry industry**

CIDC-LE
WAGEN


Familie Golsteijn boos en verdrietig over afmaken huisdier

Huiseend Winnie sterft een zachte dood

Van een verslaggeefster

BERGHEM – Boos en ver- Want hij was en is het er moest en zou dood. drietig is Jos Golstein. Eend niet mee eens dat een ge- „Schandalig", noemt een Winnie, al ge onafscheidelijk van dochter Iri avond overled heeft een spui van een dierenz zachte dood ge Golsteijn. „Er w onderuit te keer hadden d mers al gebe Berghemse ger eend Winnie n worden vanwe pest. Maar werkte steeds

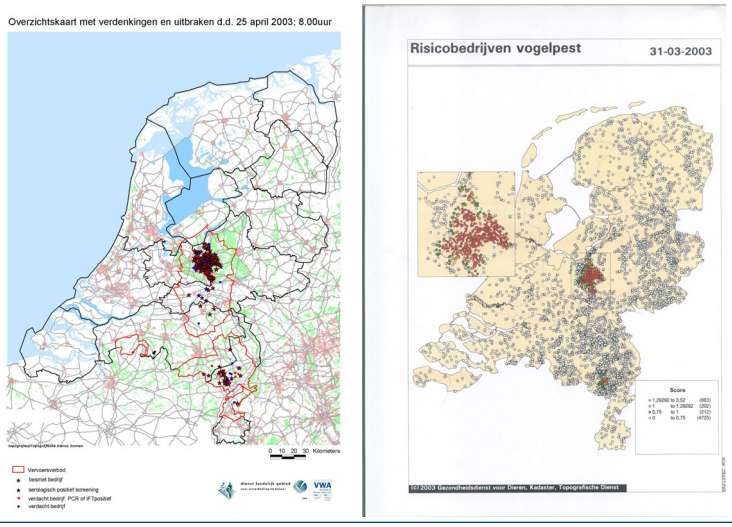
CIDC-LELYSTAD
WAGENINGEN UR

PROBLEMS OF CURRENT CONTROL

- Influenza viruses are to stay in wild birds
- Diagnosis difficult because of characteristic clinical signs.
- High density.

CIDC-LELYSTAD WAGENINGEN UR

OUTBREAKS VS. FARMS AT RISK IN THE



CIDC-LELYSTAD WAGENINGEN UR

PROBLEMS OF CURRENT CONTROL

- Diagnosis difficult because of characteristic clinical signs.
- High density.
  - any farms already infected before the outbreak is confirmed.
  - High culling capacity required
- Tracing is ineffective.
  - Many and intensive contacts
- Forced ventilation
  - High dust production

CIDC-LELYSTAD WAGENINGEN UR

WHAT CAN WE DO TO PREVENT NEW OUTBREAKS?

- Influenza viruses are to stay in the wild bird population
- Drastic decrease of density of poultry farms
  - Culling capacity adapted to density
  - Emergency vaccination
- Keep poultry indoors
- Compartmentalisation of the poultry industry
- Keeping up logbooks
- Better hygiene

CIDC-LELYSTAD WAGENINGEN UR

De Insekse bevelligingscentra worden ook nog vanuit de lucht gebrolden maar ook dikgen soldaten met tanks de steden binnen en dondert de artillerie.

Militairen aan front van vogelpest

NEDERWEERT In het Noord-Limburgse Dopel en Nederweert zijn afgelopen weekeinde negentien pluimveebedrijven gemeld, omdat er in het gebied een ernstige verdenking bestaat van vogelpest. Naast een vermoeden van het virus bij een bedrijf in Dopel, kwam er zaterdag nog een tweede verdenking bij van een kalkoenhouderij in het buurtschap Nederweert Eind. Bij de ruimingen zijn uit voorzorg tienduizenden dieren gedood. Het ministerie van Landbouw gaf opdracht tot het ruimen in een straal van 3 kilometer rondom de verdachte boerderijen, beiden in de gemeente Nederweert.

Om de ruimingen in goede banen te leiden, zette het ministerie honderd soldaten in. Zij controleren 24 uur per dag in ploegendiensten op overtredingen van de maatregelen tegen de vogelpest.

Het gaat om tien leden van de Koninklijke Marechaussee en negentig militairen van de marine, de landmacht en de luchtmacht. Zij blijven zeker tot vandaag. Over de inzet van de militairen ontstond zaterdagochtend enige opheff. Burgemeester C. Suttorp van Nederweert was boos omdat de komst van soldaten uitbleef ondanks toezeggingen van minister Veerman van Landbouw. (ANP)

CIDC-LELYSTAD WAGENINGEN UR

Military at the front of fowl plague

AN UPDATE ON AVIAN INFLUENZA IN ITALY BETWEEN 2002 AND 2003: FEATURES, CONTROL STRATEGIES AND ERADICATION OF THE H7N3 LPAI EPIDEMIC

Ilaria Capua[1], Stefano Marangon[2], Giovanni Cattoli[1], Calogero Terregino[1], Manuela dalla Pozza[2] & Lebana Bonfanti[3]

[1]OIE and National Reference Laboratory on Avian Influenza and Newcastle Disease Istituto Zooprofilattico Sperimentale delle Venezie, [2]Centro Regionale per l'Epidemiologia Veterinaria (CREV) - Istituto Zooprofilattico Sperimentale delle Venezie, Via Romea 14/A 35020, Legnaro (PD), Italy

[3]*Servizio Veterinario, Regione Lombardia, Via Pola 9, Milano- Italy*

Introduction

Between 1999 and 2001 North-eastern Italy was affected by four subsequent waves of avian influenza caused by a type A influenza virus of H7N1 subtype. The first epidemic wave was caused by a virus of low pathogenicity, which subsequently mutated to a highly pathogenic (HPAI) virus of the same subtype. The HPAI epidemic caused directly or indirectly the death or culling of over 16 million birds (1). Following eradication, the formerly HPAI infected areas were restocked. Four months after the stamping out of the last outbreak, LPAI re-emerged twice, thus determining the poultry industry to request and obtain, through the Italian veterinary authorities, vaccination against avian influenza of the H7 subtype.

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) gro
up. 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 campaign lasted for 18 months and was associated to a coordinated territorial strategy aimed at establishing whether the field virus was still circulating, and ultimately resulted in the eradication of infection.

During the month of August 2002, serological positivity at the abattoir to an H7 virus was detected in 3 meat turkey flocks. 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. The following paper describes the clinical, pathological and epidemiological data obtained from the epidemic and reports the results of a preliminary phylogenetic analysis.

Materials and methods

Birds submitted to the laboratory were clinically inspected and necropsied. Serological and virological investigations were performed in accordance to the guidelines indicated in EU

directive 92/40/EEC (3). The haemagglutinin (H) and neuraminidase (N) subtypes of influenza A isolates were determined using polyclonal chicken antisera as described by Alexander and Spackman (4).
Nucleic acid was extracted from the viruses isolated and subjected to nucleotide sequencing in the region of the genome coding for the cleavage site of the haemagglutinin molecule as described (5). Based on the HA nucleotide sequence, a phylogenetic analysis was performed.

Results

Virus isolation yielded an influenza A virus of the H7N3 subtype of low pathogenicity (LPAI). To 9th of October 2003, 388 outbreaks have been notified.

Clinical and gross findings. In meat turkeys initially, clinical signs were absent. Subsequently instead, general symptoms such as reluctance to feed, tendency to gather and ruffled feathers were accompanied by respiratory signs. Morbility was of 100%, while mortality rates were generally low in the order of 3-7%. In turkey breeders a sharp drop in egg production (from 60% to 10%) and general signs could be seen. This clinical picture was not associated to any increased mortality.
Non-specific clinical signs were observed in the guinea fowl, broilers, broiler breeders and in layers.

On post mortem, in turkeys, the constant gross finding was acute pancreatitis. In meat turkeys this finding was associated to congestion of the lung and trachea and to airsacculitis. In breeders it was accompanied by egg yolk peritonitis. No post mortem alteration was present in any of the chickens affected.

Virological investigations. H7N3 strains were isolated in embryonated fowl's SPF eggs. 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

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 lived birds, vehicles and

staff, intensive monitoring programs). The vaccination programme was based once again on a "DIVA" strategy and 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.

Discussion

The findings reported above indicate that isolate A/ty/Italy/2002/H7N3 appears to be virus of novel introduction into the domestic poultry population of Northern Italy. The preliminary phylogenetic analysis clearly indicates that the H7 gene of the isolate is related to Italian 1999-2001 H7N1, but is not identical to it, and therefore a donation of the gene from the H7N1 virus can be ruled out. Similarly it is clear that the virus is unrelated to the A/Ck/Pakistan /95/H7N3 used as a vaccine strain used in the framework of a "DIVA" vaccination strategy.

The clinical and pathological lesions are similar, although less severe than those observed during the Italian 1999-2001 LPAI H7N1 epidemic (8). The milder clinical and pathological traits of the H7N3 infection could be related to the nature of the strain or to the lower degree of adaptation these isolates have to the domestic host.

A few considerations can be made from retrospectively analysing the experience gained in the past 6 years with avian influenza in Italy. Firstly, north-eastern Italy can definitely be considered as an area "at risk" for avian influenza infections. This is also supported by AI epidemics which have occurred in the past (9,10,11,12,13) caused by viruses of the H6 and H9 subtypes. 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.
The control of LPAI infections in DPPA is a challenging experience. A coordinated set of control measures including the application of adequate biosecurity measures, the enforcement of restriction policies to restocking and movement of live birds, vehicles and staff, the implementation of a vaccination programme and of intensive monitoring measures in the

areas at risk of infection, may have different outcomes on the basis of a series of variables. These include primarily the biological characteristics of the strain, the animal species and density at the moment of AI introduction and the functional organisation of both the poultry industry and the veterinary services in the area. However, the availability of a well-structured legal basis for LPAI control, the prompt availability of vaccine, the general economic situation and the motivation of farmers and companies to eradicate the infection also play a major role in the eradication of avian influenza infections.

The experience gathered during the Italian 1997-2003 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.

In our opinion it is imperative that this disease is dealt with as a problem of the industry and of veterinary public health services. The different sets of data that are generated from surveillance and control programs at the industry level must be made available to support decision-making and this can only be achieved if there is extensive collaboration between farmers, official and field veterinarians, poultry industry, the diagnostic laboratories, the epidemiology units and the central and local governments. Only in this way it will be possible to establish a network of collaboration able to make the best of the data and tools available in the effort to control avian influenza infections in poultry.

Acknowledgments

The authors wish to thank the staff of the Epidemiology and Virology Departments of the Istituto Zooprofilattico Sperimentale delle Venezie.

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AI TEST VALIDATION

Auteur: Guus Koch
Central Institute Disease Control

CIDC-LELYSTAD WAGENINGEN UR

Contents

- RT-PCR
  - Using infection experiments
  - During outbreak
- Influenza A antigen ELISA
- Influenza A antibody ELISA
  - Using sera of infection experiments
  - Results of testing sera of the outbreak

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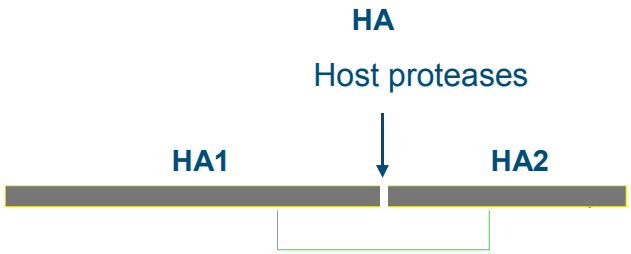
Virulence factor avian influenza A virus



Haemagglutinin (HA)
Mol. Weight: 75,000-80,000 Da

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RT-PCR used to detect first farm

- Degenerated primers to detect all H7 strains
  - GK-H7-980C (deg)
    - 5'-GYCCNMRRTATGTNAAACA-3'
  - GK-H7-1165R (deg)
    - 5'-TTRTARTCMGCMGCAGTTCC-3'
- Dilution of allantoic fluid:
  - RT-PCR compared to virus isolation 10-100 times less sensitive

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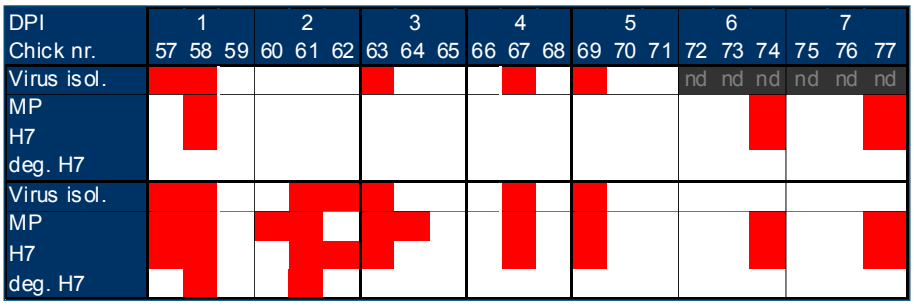
Evaluation of RT-PCR using infection experiment



- Infected with A/Ch/It/1067/V99 H7N1
  - Intratracheally and intranasally with $10^{3,5}$ EID_{50}

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PERFORMANCE OF H7 RT-PCR

		Virus Isolation	
		+	-
RT-PCR	+	218	2
	-	2	123
	%	Confidence Limits (%)	
Sensitivity	99.1	97.8 – 100	
Specificity	98.4	96.2 – 100	
Predictive value	99.1	97.8 – 100	

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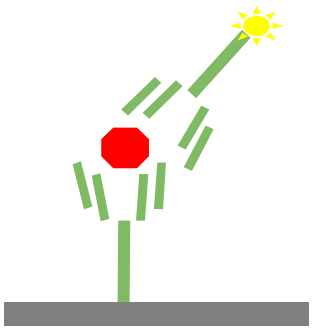
INFLUENZA A VIRUS ANTIGEN ELISA

MAb against nucleoprotein
Influenza virus H7N1
Conjugated MAb



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Performance using trachea

		VI/PCR		Total
		+	-	
ELISA	+	191	3	194
	-	25	70	95
Total		216	73	289
		%	%	
Sensitivity		88,4	84,2 – 92,7	
Specificity		95,8	91,3 – 100	
Predictive value +		98,4	96,7 – 100	
Predictive value -		73,7	74,8 – 82,5	

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Performance using trachea and lung

		VI/PCR		Total
		+	-	
ELISA	+	201	13	214
	-	18	57	75
Total		219	70	289
		%	Confidence limits %	
Sensitivity		91,8	88,1 – 95,4	
Specificity		81,4	72,3 – 90,5	
Predictive value +		93,9	90,7 – 97,1	
Predictive value -		76,0	66,3 – 85,7	

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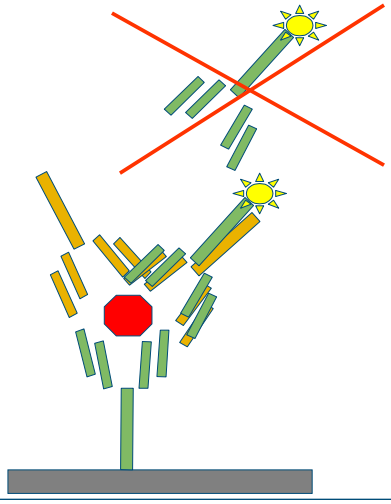
INFLUENZA A VIRUS ANTIBODY ELISA

- MAb against nucleoprotein
- Influenza virus H7N1
- Conjugated MAb
- Antibody against influenza nucleoprotein



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Infection experiment

Positive sera

- Layer or broiler type chickens of the Centre for Poultry Research
- infected with LP strains A/Ch/Penn/21525/83 H5N2 or with A/Ch/It/1067/V99 H7N1
  - Infected with 10^5 EID_{50} i.n. and i.t., 5.10^5 EID_{50} i.m.
- N=75 each
- Samples at day 10 or 11 and 14.

Negative sera

- 150 field samples with no known exposure to influenza A viruses.

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Haemagglutination inhibition test

		Infected		
		+	-	Total
HAI	+	140	0	140
	-	9	150	159
	Total	149	150	299

	%	CI %
Sensitivity	93,9 %	90,1 - 97,8 %
Specificity	100 %	91,9-100 %
Pred. Val. +	100	100 - 100 %
Pred. Val. -	94,3	90,7 – 97,9

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HI test using 'heterologous' antigen

		Infected		
		+	-	Total
HAI A/tern/H5N3	+	12	0	12
	-	60	150	210
	Total	72	150	222

	%	CI %
Sensitivity	16,7 %	8,0 – 25,3 %
Specificity	100 %	91,9-100 %
Pred. Val. +	100	100 - 100 %
Pred. Val. -	71,4	65,3 – 77,5

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INFLUENZA A VIRUS ANTIBODY ELISA

		Infected		
		+	-	Total
ELISA	+	295	6	301
	-	2	144	146
	Total	297	150	447

Sensitivity	99,3 %	98,1-100 %
Specificity	96 %	91,9-100 %
Pred. Val.	98 %	95,9-100 %

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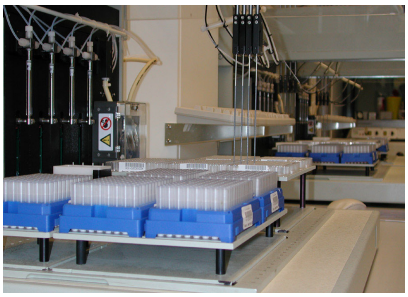
RAW RESULTS OF ANTIBODY DAS-ELISA



Total number	Number negative	Number positive	Number Non-specific	Number tested in HAI
121835 100%	108716 89,2 %	6678 5,5%	6441 5,3%	13119 10,8%

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HAI results of farms with sera positive in the ELISA

Type of flock	Number of samples	ELISA	HAI A/Dk/H5N2
Goose breeder	20	20	19
Broiler breeder	20	19	4 (12)
Goose layer	60	45	21 (22)
Zoo	61	48	5 (14)

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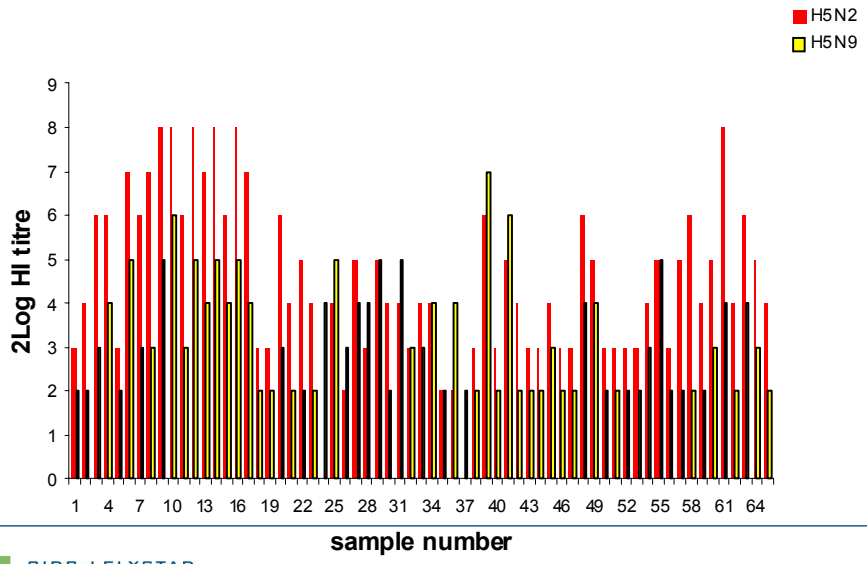
HI titre using different antigens



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Comparison of different ELISA tests

ELISA	Sensitivity (%)	Specificity (%)	Predictive value+	Predictive value -
A	97.9 96-99.9	100 100-100	100 100-100	97.4 94.9-99.9
B	85 80.0-90.1	100 100-100	100 100-100	83.5 78.0-89.0
C	91.2 87.2-95.2	99.3 98.0-100	99.4 98.3-100	89.6 84.8-94.3
D	73,0 66.8-79.3	82.3 76.1-88.5	84.4 78.9-89.9	69.9 63.1-76.8

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Conclusions

- RT-PCR can be used as an alternative for virus isolation
  - Design of primers to be adapted to sequence of isolate
- Guidelines should recommend the use of an ELISA for pre-screening of sera
  - Positive sera to be confirmed in the HI test using H7 and H5 antigens

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Closing remarks

- Standardisation of antigen and reference sera to be used?
- PCR to be incorporated in Annex of guidelines

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A COMPETITION NP-BASED AVIAN INFLUENZA ELISA TEST FOR THE DETECTION OF ANTIBODIES IN DUCK SERA

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AFSSA-Ploufragan, Avian and Rabbit Virology, Immunology and Parasitology Unit, B.P. 53, 22440 PLOUFRAGAN, France

Since the AGID test is not enough sensitive in detecting avian influenza antibodies in duck species, there is the need, for surveillance purpose, of a group specific test that can detect any AIV subtype. This is the reason why we set up a competition NP-based AI ELISA test, by using a NP specific monoclonal antibody (Mab) commercially available.

Briefly microplates were coated with either a crude inactivated AIV grown in allantoïc cavity or a control antigen (uninfected allantoïc fluid) diluted in carbonate buffer. The serum to be tested was analysed in duplicate with either antigen, after having been mixed with the NP Mab. Then a phosphate alkaline conjugate and nitrophenyl phosphate as the substrate were employed to measure the level of inhibition of the NP-Mab fixation.

The threshold of the test was calibrated using more than one hundred of sera from SPF Muscovy ducks (MD) and quarantined Peking ducks maintained in our biosafety level 3 facilities. The specificity was first assessed using around 100 sera from experimentally AIV infected SPF MD on the one hand and reference monospecific sera from MD hyperimmunized against 10 viral and 6 bacterial relevant pathogens on the other hand. Further assessment of the specificity was checked using around 250 sera from conventional MD breeders maintained in our experimental facilities. The repeatability and linearity of the test were also demonstrated. With respects to sensitivity, around 100 sera were analysed by comparing the competition ELISA with 4 other tests : homologous HI test, AGID, and 2 “duck-adapted “commercial ELISA tests.

Taking HI test as the gold standard, the relative sensitivity of our competition ELISA test was 93 %, whereas the AGID test and commercial ELISA tests displayed 15 %, 53 and 43 % respectively. At the same time the relative specificity was 100 % for our competition ELISA test and the AGID test, and 99-100% for the 2 modified commercial ELISA tests.

Given these satisfactory preliminary results, we applied our test to field sera collected from wild aquatic birds and sentinel Peking duck (sharing exactly the same living conditions as wild birds) during autumn-winter seasons from 2000-2002. By this means, mallards and sentinels displayed 62.2 and 40.5% positivity respectively. Since previous results obtained by virus isolation from cloacal samples from the same birds displayed 2.6 and 1.6 % viral positivity, we could assume this competition ELISA test detected ancient infection. However, when we attempted to analyse by HI test around 200 sera from free-range commercial ducks that were found positive by the competition ELISA test, we could not detect any positive serum using all the H1-H15 reference antigens recommended by the CRL. Therefore, if the specificity of the competition ELISA test is not implicated, the question of updating HI antigens is raised. The recent demonstration of the reference H5 antigen defect (using european ring test sera) gives support to the second hypothesis.

Acknowledgement

The authors want to acknowledge ONCFS (the organism for wild animals and hunting from the French ministry of environment) particularly Dr J. HARS for the financial and logistic support for collecting samples from wild birds and sentinels, and the French ministry of agriculture for the financial support for implementing the competition ELISA test

COMPARISON OF THREE RAPID DETECTION SYSTEMS FOR TYPE A INFLUENZA VIRUS ON TRACHEAL SWABS OF NATURALLY AND EXPERIMENTALLY INFECTED BIRDS

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INTRODUCTION

Influenza A viruses infecting poultry can be divided into two distinct groups on the basis of their ability to cause disease. The very virulent viruses cause highly pathogenic avian influenza (HPAI) in poultry, which may result in flock mortality as high as 100%. These viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI. All other viruses cause a much milder disease consisting primarily of mild respiratory disease, depression and egg production problems in laying birds (Low Pathogenicity Avian Influenza, LPAI). Both HPAI and LPAI are highly contagious diseases, able to spread in a susceptible population in a short period of time. Therefore, the prompt identification of an infected flock is crucial for eradication purposes, since it enables the enforcement of restriction policies.

For diagnostic purposes the selection of proper samples and test methods is of fundamental importance in order to make diagnosis rapid and reliable. Virus isolation (VI) in specific pathogen free (SPF) fowl's eggs from swabs or pathological samples is considered the most reliable means of establishing whether a flock is infected or not. However it is a laborious and time consuming assay, and the sample processing times are often not compatible with the demands of the complex organisation of the poultry industry. In addition, the delay in moving birds from a premise, whilst awaiting for the VI result, often also results in animal welfare issues. For this reason, when dealing with an epidemic of AI, rapid and reliable laboratory tests should be available to reveal direct evidence of infection in the flocks located in the areas at risk of infection.

In the present study samples collected during the H7N3 subtype influenza A virus epidemic occurred in Italy in 2002-2003 and following an experimental infection were used to assess the relative sensitivity and specificity of a commercial antigen capture enzyme immunoassay (AC-EIA) and of two nucleic acid detection tests comparing them to the VI test. Their suitability for diagnostic purposes during an epidemic was evaluated.

MATERIALS AND METHODS

Experimental infection

40 commercial turkeys, hatched in isolation, were divided randomly in to 4 groups of 10. Prior to challenge all birds were serologically and virologically tested and shown to be negative to antibodies to type A influenza virus. The challenge virus was a LPAI isolate of the

H7N3 subtype (A/ty/Italy/8000/02) obtained in Italy during the 2002-2003 epidemic. The challenge virus was titrated in SPF eggs, diluted in sterile PBS solution to obtain concentrations of 10^2, 10^4 ,10^6 EID_{50}/100µl and used to intranasally infect three experimental groups. The remaining 10 birds were left as uninoculated controls. Tracheal swabs were collected on days 3, 5, 7, 10, 12, 15 and 20 and processed for AC-EIA, virus isolation, one step RT-PCR and real-time PCR (RRT-PCR). Birds were bled on day 20 and sera were tested for antibodies to the H7 subtype of AI using the haemagglutination inhibition test (HI) (CEC, 1992).

Field samples

On 232 poultry farms included in the monitoring area ten birds were selected at random and swabbed. Tracheal swabs were collected by official and field veterinarians and submitted to the laboratory within 24 hours.

Sample handling, serology and virology

Tracheal swabs collected from the experimental and field studies were pooled (10 swabs/pool) and suspended in 1 ml of sterile phosphate buffered saline (PBS, pH 7.2).
All serological and virological investigations were performed according to EU Directive 92/40/EEC (CEC 1992).

Antigen detection

A commercially available AC-EIA kit (Directigen®™, Becton Dickinson) was used for the detection of type A antigen on tracheal swabs according to the manufacturer's instructions.

RNA detection tests

Two hundred µl of PBS suspension were used to extract the RNA using a commercial kit (High Pure™ RNA extraction kit, Roche). For the RRT-PCR, 30 µl of RNA solution were retrotranscribed with random hexamers and published primers targeting the M gene of type A influenza virus were applied for PCR, namely forward primer M+25 and reverse primer M -124 (Spackman *et al.,* 2002) at the optimised concentration of 300 nM each. cDNA was amplified in a final volume of 25 µl using the SYBR®GREEN PCR Master Mix (Applied Biosystems). The PCR reaction was performed in a ABI Prism 7700 SDS apparatus (Applied Biosystems). After the PCR cycles, a DNA melting curve was generated in order to discriminate between specific amplicon and non-specific amplification products (Ririe *et al*., 1997). Forward primer M52C and reverse primer M253R (Fouchier *et al*., 2000) targeting the M gene of type A influenza virus were applied for one-step RT-PCR. The one-step RT-PCR was performed in a 9700 Thermalcycler (Applied Biosystems).

Analytical specificity and sensitivity

The primer sets tested in the one-step PCR and real time RT-PCR protocol were used to amplify the RNA of type A influenza virus belonging to different subtypes (from H1 to H15) and other common avian viral pathogens, namely Newcastle Disease and Infectious Bronchitis viruses, in order to assess the specificity and the ability to amplify a broad range of type A influenza strains. In addition, the challenge virus was diluted in sterile PBS solution to obtain titres ranging from 10^7 to 10^{-2} EID_{50}/100µl. Each dilution was tested by the AC-EIA and two nucleic acid detection methods to assess the analytical sensitivity of the diagnostic protocols.

RESULTS

Experimental infection

The results of the experimental infections are summarised in Table 1. The results indicate that in experimentally infected turkeys challenged with 10^4 and 10^6 EID_{50}/100µl, virus isolation was positive from the pooled tracheal swabs collected from day 3 to day 10. One-step RT-PCR was able to detect influenza RNA from samples collected from day 3 to day 12, while RRT-PCR amplified influenza RNA in swabs collected from day 3 to day 15. The AC-EIA test yielded positive results between day 5 and 10 post-infection. The uninoculated controls and the group challenged with 10^2 EID_{50} remained negative for the duration of the experiment and did not show any evidence of seroconversion. The results of the serological investigation on day 20 post-infection indicated that infection was achieved in the group challenged with 10^4 and 10^6 EID_{50} in 5/10 birds and 10/10 birds respectively.

Field samples

Generally speaking, comparison between virus isolation, the AC-EIA test and the two nucleic acid detection methods indicated excellent agreement and the results derived from the field samples are summarised in table 2, 3 and 4 respectively. The K value between the AC-EIA and VI tests was 0.82. Compared to virus isolation, the relative sensitivity of this test was 88.9% (CI_{95}=85.2-92.6) and the relative specificity was 95.7% (CI_{95}=93.7-97.7). The K value between the RT-PCR and VI tests was 0.88. Compared to virus isolation, the relative sensitivity of the one-step RT-PCR was 95.6% (CI_{95}=93.1-98.0) and the relative specificity was 96.3% (CI_{95}=94.4-98.1). The K value between the RRT-PCR and VI tests was 0.92. Compared to virus isolation, the relative sensitivity and specificity of RRT-PCR was 93.3% (CI_{95}=90.4-96.3) and 98.4% (CI_{95}=97.2-99.6) respectively.

Analytical specificity and sensitivity

By both one step RT-PCR and RRT-PCR a specific amplification product was obtained with all the type A influenza strains tested. The cDNA of the NDV and IBV strains was not amplified by these methods. The detection limit of the RRT-PCR protocol was determined to be approximately equivalent to 1 EID_{50}. For the one step RT-PCR and the AC-EIA method the detection limit was determined to be approximately equivalent to 10^2 and 10^4 EID_{50} respectively.

DISCUSSION

In the present report, comparison between virus isolation, the AC-EIA test and the two nucleic acid detection methods showed excellent agreement. Data obtained from both experimental and field study indicate a higher sensitivity of the PCR based methods compared to the AC-EIA. The analytical sensitivities of the RRT-PCR and the one-step RT-PCR, were 10,000 and 100 –fold greater than the AC-EIA respectively. Our results confirm previous studies (Fouchier *et al.,* 2000; Spackman *et al*., 2002) and these methods appeared to be specific and able to amplify cDNA from a large variety of influenza strains belonging to different HA subtypes.

The ability of a diagnostic test to detect evidence of AI infection during the early phases is crucial for the implementation of eradication procedures during an epidemic. Virus isolation results obtained from the experimentally infected turkeys demonstrated that infectious virus

was present in the trachea from day 3 to day 10 post-challenge. In these birds the nucleic acid detection methods were more sensitive than the AC-EIA. AC-EIA was able to detect infection from day 7 to day 10 or from day 5 to day 7 post-challenge, depending on the infectious dose, while PCR methods were positive from day 3 to day 15 or 12 post-challenge. The better performances of the PCR based methods, in terms of relative sensitivity and specificity, were also observed during the field study.

The sensitivity of the AC-EIA recorded with the field samples could be explained by the data obtained from the analytical sensitivity assay. The higher amount of virus needed for the test to result positive is probably only present for a limited number of days following natural infection. These results are in accordance with previous studies where the same AC-EIA test was applied to samples of avian, swine and equine origin. In those studies a relative sensitivity values ranging between 79 % and 86 % were obtained (Ryan-Poirier *et al.*, 1992; Chambers *et al*., 1994; Davison *et al*., 1998).

Overall, RRT-PCR appears to be rapid and cost-effective, a valuable assay for testing very large number of samples as in the management of an epidemic. However, it requires expensive equipment and for this reason has the limitation of not being available in peripheral laboratories. The one step RT-PCR is instead suitable for local laboratories primarily involved in monitoring: large numbers of samples can be processed and no expensive equipment is necessary. However, in the management of AI epidemics it occurs that some samples need to be processed urgently. In this case, the AC-EIA test remains the method of choice. Although each laboratory must determine the most suitable testing methods, we feel that the nucleic acid detection methods described are a valuable tool for AI diagnosis in emergency situations and they should replace antigen detection tests during monitoring and screening involving large numbers of samples.

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Table 1. Comparison between different methods for the detection of AI (virus, antigen or RNA) in tracheal swabs of turkeys experimentally infected with LPAI A/ty/Italy/8000/03 H7N3 at different infectious doses.

Methods	EID_{50}	*AI detection from pooled tracheal swabs (10 birds/pool)*						
RRT-PCR	10^2	-	-	-	-	-	-	-
One step RT-PCR	10^2	-	-	-	-	-	-	-
AC-EIA	10^2	-	-	-	-	-	-	-
Virus Isolation	10^2	-	-	-	-	-	-	-
Day post-infection		**3**	**5**	**7**	**10**	**12**	**15**	**20**
RRT-PCR	10^4	+	+	+	+	+	+	-
One step RT-PCR	10^4	+	+	+	+	+	-	-
AC-EIA	10^4	-	-	+	+	-	-	-
Virus Isolation	10^4	+	+	+	+	-	-	-
Day post-infection		**3**	**5**	**7**	**10**	**12**	**15**	**20**
RRT-PCR	10^6	+	+	+	+	+	+	-
One step RT-PCR	10^6	+	+	+	+	+	-	-
AC-EIA	10^6	-	+	+	-	-	-	-
Virus Isolation	10^6	+	+	+	+	-	-	-
Day post-infection		**3**	**5**	**7**	**10**	**12**	**15**	**20**

EID_{50} = Egg infectious dose of challenge virus
RRT-PCR = real time PCR
AC-EIA= antigen capture enzyme immunoassay
+ = positive
- = negative

Table 2. Comparison between virus isolation and the AC-EIA test

	VI positive	VI negative	Total AC-EIAsamples
AC-EIA positive	**40**	8	48
AC-EIA negative	5	**179**	184
Total VI samples	45	187	**232**

Table 3. Comparison between virus isolation and one step RT-PCR tests

	VI positive	VI negative	Total RT-PCR samples
one step RT-PCR positive	**43**	7	50
one step RT-PCR negative	2	**180**	182
Total VI samples	45	187	**232**

Table 4. Comparison between virus isolation and RRT-PCR tests

	VI positive	VI negative	Total RRT-PCR samples
RRT-PCR positive	**42**	3	45
RRT-PCR negative	3	**184**	187
Total VI samples	45	187	**232**

Lab organisation and sample processing during Dutch HPAI outbreak

Author: Guus Koch

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Central Institute for Animal Disease Control - Lelystad

- National Reference Institute
  - Surveillance monitoring, safeguarding trade flows
  - Management and safety supervision HCU
- Prevention and control of notifiable and infectious animal diseases
  - Animal and public health, food safety
  - Including: AI, CSF, FMD, BSE and Anthrax
  - Diagnostics, epidemiology, emergency vaccines, specific diagnostics,
  - Safeguarding emergency stock FDM vaccine
- Assessment and control of veterinary medicinal products and feed additives
  - Advice on registration
  - Advice on problem solving
  - Quality control of immunological veterinary products

CIDC-LELYSTAD
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Main processes of CIDC-Lelystad

1. Diagnostic research & expertise maintenance*
2. Scientific research
3. Advice*
4. Supervision of diagnostics in private laboratories
5. Crisis organisation (*component)

CIDC-LELYSTAD WAGENINGEN UR

Crisis organisation

- 24 hours 7 days per week
- Contingency plans + exercises
- Advice and diagnostics during a crisis
  - 1994: SVD
  - 1997/98: CSF
  - 2001: BSE
  - 2001: FMD
  - 2001: Anthrax
  - 2003: AI

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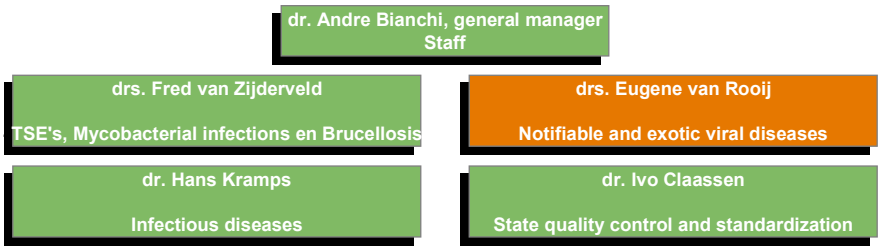
Organisation chart CIDC-Lelystad



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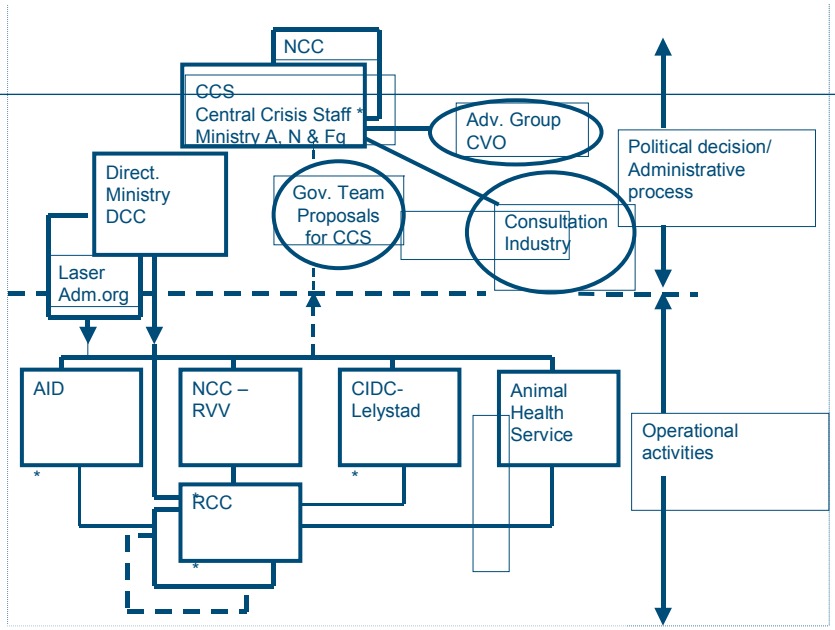

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Contingency plan

- Internal process
  - Internal crisis team
    - Responsibilities & competences
  - Rooms and equipment
  - Supplies of materials, reagents, etc.
  - People and training
  - Trajectory of samples and results

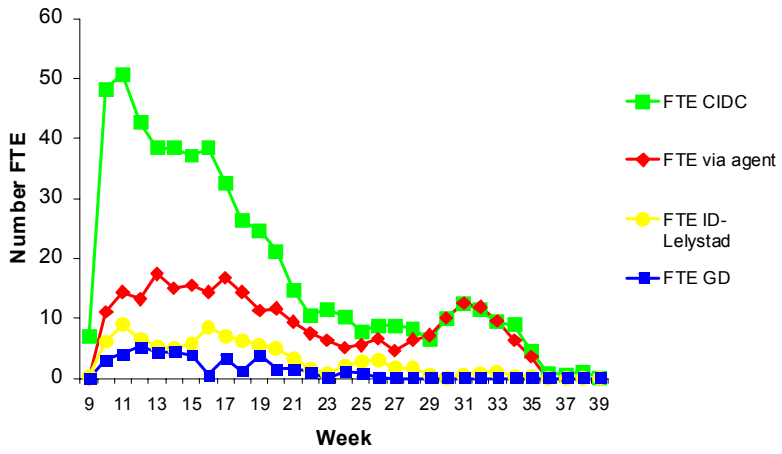
CIDC-LELYSTAD WAGENINGEN UR

Contingency plan

- Director decides for implementation of crisis team and appoints manager crisis team (outbreak AI nearby?)
- Crisis manager convenes the meeting of the crisis team

CIDC-LELYSTAD WAGENINGEN UR

Personal per week

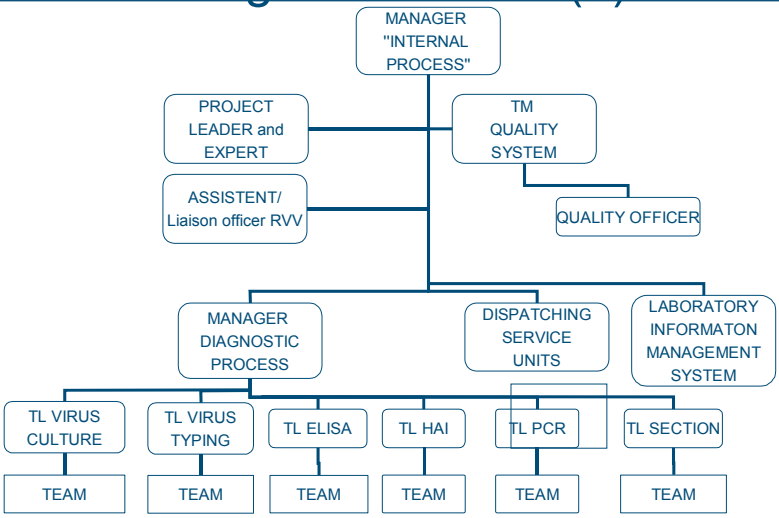


CIDC-LELYSTAD WAGENINGEN UR

Crisis management CIDC (1)

CIDC-LELYSTAD WAGENINGEN UR

Crisis management CIDC (2)



CIDC-LELYSTAD WAGENINGEN UR

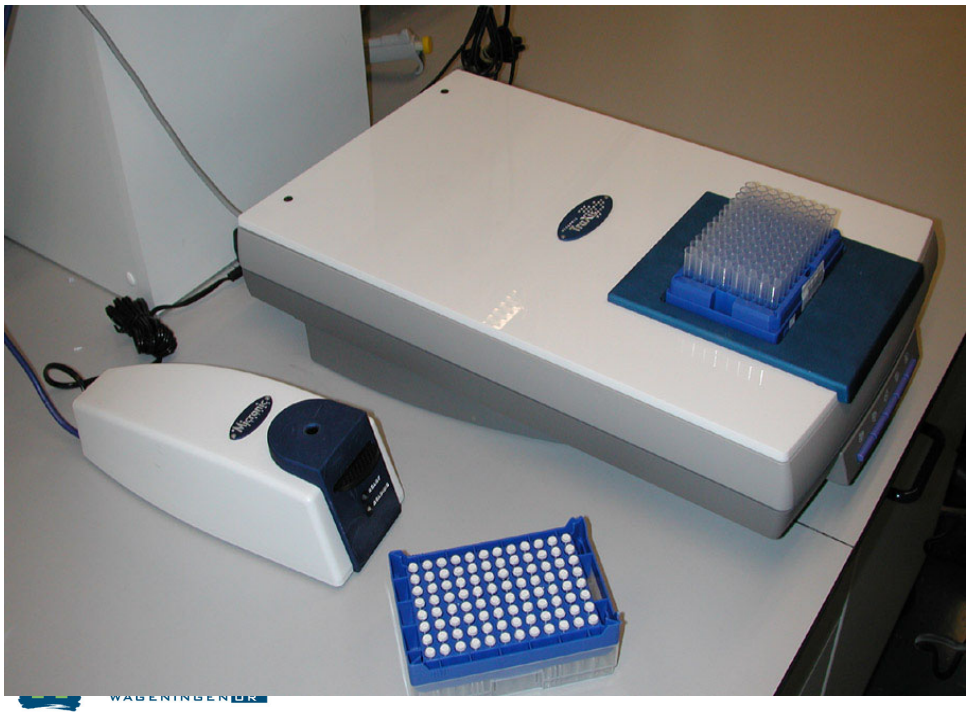
CIDC-LELYSTAD WAGENINGEN UR

Sample administration upon arrival

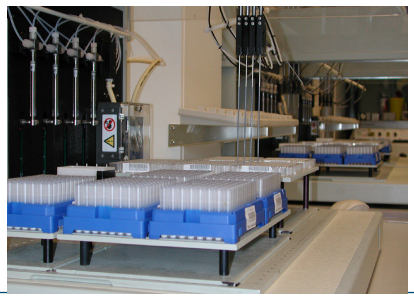
CIDC-LELYSTAD
WAGENINGEN UR

Tracing-tracking system

CIDC-LELYSTAD
WAGENINGEN UR



ELISA



CIDC-LELYSTAD
WAGENINGEN UR

Test results

- Test results:
  - Are entered in defined manner in LIMS
  - Are authorised by a staff member in LIMS
  - Are send by LIMS via E-mail to State National Crisis Centre
- CVO and director of RVV are warned about infected farms by phone

CIDC-LELYSTAD WAGENINGEN UR

Thanks for your attention

CIDC-LELYSTAD WAGENINGEN UR

INCREASED RESISTANCE OF VACCINATED TURKEYS TO EXPERIMENTAL INFECTION WITH AN H7N3 LOW PATHOGENICITY AVIAN INFLUENZA VIRUS

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INTRODUCTION

Between 1997 and 2003 North-eastern Italy was affected by 7 introductions of avian influenza viruses of the H5 or H7 subtypes. Some of the introductions resulted in spread of the virus among the industrial poultry population, while others were of limited relevance for the poultry industry.
In order to support the eradication of two of these viruses, namely LPAI H7N1 (2000-2002) and LPAI H7N3 (2002-2003), a "DIVA" vaccination strategy was developed and applied in the field. The DIVA strategy is based on using an inactivated vaccine with a homologous haemagglutinin to the field virus and a diverse neuraminidase. Basically antibodies to the neuraminidase protein of the field are used as a "natural marker" of infection.
Vaccination with inactivated vaccines exhibiting a diverse neuraminidase have been shown to be cross-protective and to reduce the shedding levels., however, no data are available on whether vaccinated birds are less susceptible to infection. If this was the case, the coupled effect of reducing the viral load in the environment and the reduction of susceptibility to field challenge would represent valid reasons to promote vaccination programs during eradication campaigns, particularly in densely populated poultry areas (DPPA). The aim of the present study was to establish the degree of susceptibility and virus shedding in turkeys vaccinated with a heterologous influenza strain to that of the challenge virus.

MATERIALS AND METHODS

Birds

60 commercial turkeys, hatched in isolation, were divided randomly in 2 groups of 30. One group was vaccinated as described below. The remaining group was left as uninoculated controls. Each group was subsequently divided into 3 groups of 10. They were reared in isolation with feed and water *ad libitum.*

Vaccine

Commercially available inactivated oil-emulsion vaccine containing the A/ty/Italy/99/ H7N1 strain.

Challenge virus

The challenge virus was a LPAI isolate of the H7N3 subtype (A/ty/Italy/8000/02) obtained in Italy during the 2002-2003 epidemic.

Experimental design

One group of ten vaccinated and one group of ten control birds were infected with one of each viral suspension containing 10^2, 10^4 ,10^6 EID_{50}/ 100µl respectively. Each bird received 100µl intranasally.

Birds were inspected clinically twice a day. Tracheal swabs were collected and pooled on days 3, 5, 7, 10, 12, 15 and 20 and processed for antigen detection (ELISA), by commercially available kit (Directigen®™, Becton Dickinson) virus isolation in specific pathogen free eggs according to EU Directive 92/40EEC (CEC, 1992), and real-time PCR (RRT-PCR).

Cloacal swabs were collected on days 3, 5, 7, 10, 15 and 20 and processed for virus isolation and PCR. Prior to vaccination all birds were tested by the AGP test and shown to be negative to antibodies to group A antigen of avian influenza virus. Birds were bled on day 71 (21 days following the last vaccination) and sera were tested for antibodies to the H7 subtype of AI using the heamagglutination test (HI).

Statistical analysis

Serological titres pre and post challenge were subjected to statistical analysis to evaluate significance in seroconversion among and within experimental groups by the Wilcoxon-Mann-Whitney rank-sum test (Siegler & Castellan, 1992; Thrusfield, 1995) and non parametric Wilcoxon matched-pairs signed rank test.

RESULTS

No clinical signs were observed in any of the birds belonging to the vaccinated groups. Clinical signs were observed in the unvaccinated birds infected with 10^4, 10^6 EID_{50}/100µl. 5/10 birds challenged with 10^4 EID_{50} showed depression associated with a mild diarrhoea and respiratory signs starting on day 4 post-infection. Three birds challenged with 10^6 EID_{50} also exhibited sinusitis, characterized by swelling of the infraorbital sinuses. All clinical signs, except for the sinusitis, were self-limiting and disappeared by day 20 post-infection.

The results of virological investigations, RT-PCR and antigen detection on cloacal and tracheal swabs and the serological results are presented in tables 1, 2 and 3 respectively. Infection was not achieved in the birds challenged with 10^2 EID_{50}/100µl. From the analysis of the serological data pre and post-challenge, it appears that infection was achieved in the naïve but not in the vaccinated birds challenged with 10^4 EID_{50}. In the latter seroconversion or shedding were not detected, while in the naïve birds seroconversion and viral RNA was detected in 5 out of 10 animals. Among the infected animals, at least one was shedding live virus, as indicated by virus isolation (table 1). Infection was achieved in the birds challenged with 10^6 EID_{50} regardless of their state of vaccination. On analysing data obtained from the pooled tracheal swabs, a reduction of the duration of shedding was observed (table 2). However, taking together the results of the virus isolation, RRT-PCR and serology, a reduction in the number of infected birds was observed in the vaccinated birds (table 1). Statistical analysis performed on the serological data indicated that serological titres pre-infection did not differ significantly and therefore the vaccinated birds belonging to the three experimental groups belonged to a homogeneous population.

Serological titers in the groups challenged with 10^6 EID_{50} differed significantly (p-value <0.05) from the other two experimental groups, thus indicating that active infection was achieved with this viral load. The Wilcoxon matched pairs signed rank test was applied for the assessment of statistical significance between pre and post challenge serological titres. A

significant value(p-value <0.05) was obtained only for the group challenged with 10^6 EID_{50}/100µl.

DISCUSSION

The data presented indicate that infection was achieved only with the viral suspensions containing 10^4 and 10^6 EID_{50} in the control birds, and with 10^6 EID_{50} also in the vaccinated birds. In the groups challenged with 10^4 EID_{50}, shedding and evidence of active viral replication was only detected in the control birds. Active infection was achieved in both the naïve and in the vaccinated birds challenged with10^6 EID_{50}. A reduction of the number of birds excreting virus and of the duration of shedding in the vaccinated vs unvaccinated controls was detected by all three tests (virus isolation, RRT-PCR and ELISA) in the samples obtained from tracheal swabs. A similar result was obtained from cloacal swabs processed for attempted virus isolation. However, the latter samples were positive using the RRT-PCR test both in the vaccinated and in the unvaccinated populations up to the termination of the experiment (day 20 p.i.).
The results of the virological investigations were in agreement with the results of the serological investigations. There was no seroconversion in the vaccinated or control birds challenged with 10^2 $EID_{50,}$ thus indicating that for this particular strain of virus, a higher viral dose is necessary to achieve infection. The higher infectious dose of 10^4EID_{50} was able to induce active infection and seroconversion in the unvaccinated controls but not in the vaccinated birds.
The data presented in the present paper, indicate that the use of vaccination may be appropriate as a tool to support eradication measures employed during an AI outbreak. In addition to the well known effect on reduction of shedding of infectious virus (Swayne & Suarez, 2000) vaccination generates a higher resistance to infection. The combination of these two effects is particularly useful in areas at risk with a high density of susceptible animals.
Devastating epidemics of avian influenza have occurred recently in DPPA in Italy and in the Netherlands causing the death or culling of over 45 million birds overall. From the data presented it appears that the implementation of an appropriate "DIVA" vaccination strategy could have reduced the massive spread of infection in the DPPAs and therefore the death and culling of millions of animals.
However, that vaccination alone will not achieve the goal of eradication. Strict biosecurity measures and restriction policies represent the main tools to prevent the introduction and perpetuation of avian influenza infections in domestic poultry. Vaccination should be only considered as a tool to maximise the effect of sanitary measures in the face of an outbreak or when the risk of introduction in DPPA exists.

ACKNOWLEDGEMENTS

Mr. Serafino (Berto) Pianta is gratefully acknowledged for performing the animal experiments. We also wish to thank Sonia Fassina and Elena Bertoli for their invaluable technical assistance and Marzia Mancin for performing the statistical analysis.

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Table 1. Results of virus isolation and viral genome detection (RRT-PCR) assays from cloacal swabs of control and vaccinated birds challenged A/t/Italy/8000/V02 (H7N3) virus.

INFECTIOUS DOSE	GROUP	3 days p.c.	5 days p.c.	7 days p.c.	10 days p.c.	15 days p.c.	20 days p.c.	Total samples positive	3 days p.c.	5 days p.c.	7 days p.c.	10 days p.c.	15 days p.c.	20 days p.c.	Total number RNA positive
10^2EID_{50}	Vaccinated	0/10*	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Not vaccinated	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
10^4EID_{50}	Vaccinated	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Not vaccinated	0/10	1/10	1/10	0/10	0/10	0/10	2/10	0/10	2/10	3/10	1/10	0/10	0/10	6/60
10^6EID_{50}	Vaccinated	0/10	4/10	4/10	2/10	1/10	0/10	11/60	0/10	3/10	3/10	2/10	3/10	2/10	13/60
	Not vaccinated	0/10	5/10	6/10	3/10	1/10	1/10	16/60	0/10	5/10	9/10	4/10	2/10	1/10	21/60

* Number of turkeys positive/number of turkeys in the group (per day)

Table. 2. Results of virus isolation, antigen and viral genome detection (RRT-PCR) assays from pool of tracheal swabs of control and vaccinated birds challenged with A/ty/Italy/8000/V02 (H7N3) virus.

Infectious dose	Group	3 days p.c.	5 days p.c.	7 days p.c.	10 days p.c.	12 days p.c.	15 days p.c.	20 days p.c.
					RRT-PCR			
	Vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10^2EID_{50}/ml	Not vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	Vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10^4EID_{50}/ml	Not vaccinated	+	+	+	+	+	+	neg.
	Vaccinated	+	+	+	+	+	neg.	neg.
10^6EID_{50}/ml	Not vaccinated	+	+	+	+	+	+	neg.
					ELISA			
	Vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10^2EID_{50}/ml	Not vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	Vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10^4EID_{50}/ml	Not vaccinated	neg.	neg.	+	+	neg.	neg.	neg.
	Vaccinated	+	neg.	neg.	neg.	neg.	neg.	neg.
10^6EID_{50}/ml	Not vaccinated	neg.	+	+	neg.	neg.	neg.	neg.
					Virus isolation			
	Vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10^2EID_{50}/ml	Not vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	Vaccinated	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10^4EID_{50}/ml	Not vaccinated	+	+	+	+	neg.	neg.	neg.
	Vaccinated	+	+	neg.	neg.	neg.	neg.	neg.
10^6EID_{50}/ml	Not vaccinated	+	+	+	+	neg.	neg.	neg.

p.c. = post-challenge

Table. 3. Serological response of bird to challenge with A/ty/Italy/8000/V02 (H7N3) virus.

Infectious dose	Group	Neg.	<1:16	≥1:16	≥1:32	≥1:64	≥1:128	≥1:256	≥1:512	GMT [b]
		HI prior to challenge								
10^2EID_{50}/ml	Vaccinated	0/10[a]	0/10	10/10	10/10	9/10	5/10	3/10	1/10	**6.8**
	Not vaccinated	10/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	**0**
10^4EID_{50}/ml	Vaccinated	0/10	0/10	10/10	10/10	10/10	6/10	4/10	2/10	**7.3**
	Not vaccinated	10/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	**0**
10^6EID_{50}/ml	Vaccinated	0/10	0/10	10/10	10/10	8/10	3/10	0/10	0/10	**6.1**
	Not vaccinated	10/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	**0**
		HI post- challenge								
10^2EID_{50}/ml	Vaccinated	0/10	0/10	10/10	9/10	2/10	0/10	0/10	0/10	**5.2**
	Not vaccinated	10/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	**0**
10^4EID_{50}/ml	Vaccinated	0/10	0/10	10/10	8/10	5/10	2/10	1/10	0/10	**5.6**
	Not vaccinated	5/10	5/10	5/10	5/10	3/10	0/10	0/10	0/10	**2.8** [c]
10^6EID_{50}/ml	Vaccinated	0/10	0/10	10/10	10/10	10/10	10/10	9/10	9/10	**9.6** [c]
	Not vaccinated	1/10	1/10	9/10	8/10	8/10	8/10	3/10	0/10	**6.5** [c]

[a] number with titre/number of turkeys of the group.
[b] GMT: geometric mean titre
[c] Statistically significant difference with pre-challenge titre

Experimental Study to Assess the Risk of Low and High Pathogenicity Avian Influenza Virus for Transmission in Chicken Meat

David Swayne
Southeast Poultry Research Laboratory
USDA/ARS, Athens, GA

Introduction

Avian Influenza Viruses

- Replication needs hemagglutinin cleavage – enzyme dependent: HA0 ⇒ HA1 & HA2
  - LPAI – trypsin-like enzymes cleave HA0
    - present in limited number of cells, primarily epithelium of respiratory and gastrointestinal tracts
  - HPAI – Furin class of ubiquitous proteases cleave HA0
    - present in a large number of different cell types throughout the body

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1

Export Issues

Low Pathogenicity Avian Influenza in Meat

Mo et al., 1997 – no IHC demonstration of PA/83 (H5N2) & AL/75 (H4N6) LPAIV in chicken skeletal muscle

- Rosenberger et al., 2002 – no isolation of PA/97 (H7N2) LPAIV in chicken meat
- Yamaguchi et al., 2002 – isolated H9N2 AIV from imported chicken meat plus reproduced infection in chickens and recovery of H9N2 AIV from meat

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Introduction

Highly Pathogenic AI Virus in Meat

- Purchase, 1931– transmit HPAI by feeding blood but not meat
- Mo et al., 1997 – IHC demonstrated HPAIV in meat (Ont./66 [H5N9], PA/83 [H5N2], Vict./85 [H7N3])
- Perkins and Swayne, 2001 – demonstrated HK/97 (H5N1) HPAIV muscle of chickens
- Tumpey et al., 2002 – demonstrated Anyang/01 (H5N1) in chicken and duck meat
- Rosenberger et al., 2002 – isolation of PA/83 (H5N2) HPAIV in chicken meat

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2

Materials and Methods

- Inoculum
  - A/turkey/VA/158512/02 (H7N2) LPAIV, A/chicken/PA/21525/83 (H5N2) LPAIV and A/chicken/PA/1370/83 (H5N2) HPAIV – app. 10^6 EID_{50}/bird
  - Sterile uninfected allantoic fluid (Sham-inoculated)
- Experiment design
  - 35 SPF White Rock chickens: virus-inoculated (n=25) and sham-inoculated (n=10) groups – intranasally at 4 weeks of age
  - Sampled 5 bird: 1, 3, 5, 7 and 10 days post-inoculation (PI)
  - Samples for virus isolation: abdominal air sac swab, oral swab, cloacal swab, blood, lung, trachea, thigh meat, breast meat and bone/bone marrow (ischium)
  - Serology at 0 and 10 days PI (AGID)

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Materials and Methods

- Day 3 PI – additional virus isolations
  - Body cavity wash (3 ml)
  - Chilled in chlorine rinse water for 60 minutes at 35-37F
  - Body cavity wash (3 ml)
- Meat feed from 3 DPI to 30 SPF WPR chickens – 14 days later check for seroconversion to AIV
  - Sham chickens (n=10) – combined breast and thigh meat (50-100 gm total)
  - Virus-inoc. (3 DPI) breast meat (n=10) (50 gm total)
  - Virus-inoc. (3 DPI) thigh meat (n=10) (25 gm total)

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3

Results - Initial

Virus Isolation: H7N2 LPAIV

		Swabs		
Group	Date Sampled	Oral	Cloacal	Air Sac
Sham-inoc.	3	0/5	0/5	0/5
	10	0/5	0/5	0/5
Virus-inoc.	1	5/5	0/5	0/5
	3	5/5	0/5	0/5
	5	5/5	1/5	0/5
	7	0/5	0/5	0/5
	10	0/5	0/5	0/5

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Results - Initial

Virus Isolation (H7N2) LPAIV – 3 DPI

	Blood		
Group	Plasma	WBC	RBC
Sham-inoc.	0/5	0/5	0/5
Virus-inoc.	0/5	0/5	0/5

	Tissues						
Group	Trachea	Lung	Bone	Breast Meat	Thigh Meat	Pre-Chill Rinse	Post-Chill Rinse
Sham-inoc	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Virus-inoc.	3/5	3/5	0/5	0/5	0/5	0/5	0/5

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4

Results - Initial

Virus Isolation: H5N2 LPAIV

		Swabs		
Group	Date Sampled	Oral	Cloacal	Air Sac
Sham-inoc.	3	0/5	0/5	0/5
	10	0/5	0/5	0/5
Virus-inoc.	1	5/5	0/5	0/5
	3	5/5	2/5	0/5
	5	5/5	4/5	2/5
	7	3/5	3/5	0/5
	10	0/5	0/5	0/5

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Results - Initial

Virus Isolation (H7N2) LPAIV – 3 DPI

	Blood		
Group	Plasma	WBC	RBC
Sham-inoc.	0/5	0/5	0/5
Virus-inoc.	0/5	0/5	0/5

	Tissues						
Group	Trachea	Lung	Bone	Breast Meat	Thigh Meat	Pre-Chill Rinse	Post-Chill Rinse
Sham-inoc	0/5	0/5	0/5	0/5	0/5	?	?
Virus-inoc.	2/5	2/5	0/5	0/5	0/5	?	?

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5

Results - Initial

Virus Isolation: H5N2 HPAIV

		Swabs		
Group	Date Sampled	Oral	Cloacal	Air Sac
Sham-inoc.	3	0/5	0/5	0/5
	10	0/5	0/5	0/5
Virus-inoc.	1	5/5	1/5	0/5
	3	5/5	5/5	3/5
	5	5/5	4/5	4/5
	7	NA	NA	NA
	10	NA	NA	NA

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Results - Initial

Virus Isolation (H5N2) HPAIV – 3 DPI

	Blood		
Group	Plasma	WBC	RBC
Sham-inoc.	0/5	0/5	0/5
Virus-inoc.	0/5	3/5	4/5

	Tissues						
Group	Trachea	Lung	Bone	Breast Meat	Thigh Meat	Pre-Chill Rinse	Post-Chill Rinse
Sham-inoc	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Virus-inoc.	5/5	5/5	4/4	4/4	5/5	5/5	5/5

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6

Results

Meat Feeding Experiment

- Three groups:
  - Sham meat group (breast and thigh)
  - Breast meat of 2 LPAI & 1 HPAI groups
  - Thigh meat of 2 LPAI & 1 HPAI group
- 14 day observation period
  - No clinical signs
  - Negative for antibodies (AGID)

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Summary

- Using a natural route of exposure, i.e. IN:
  - Two LPAIV caused a predominate respiratory, less so in intestine, infection with all birds in the virus-inoculated group having virological and/or serological evidence of infection
  - LPAIV was not recovered from thigh or breast meat
- Feeding breast or thigh meat from LPAIV-inoculated chickens did not transmit AI

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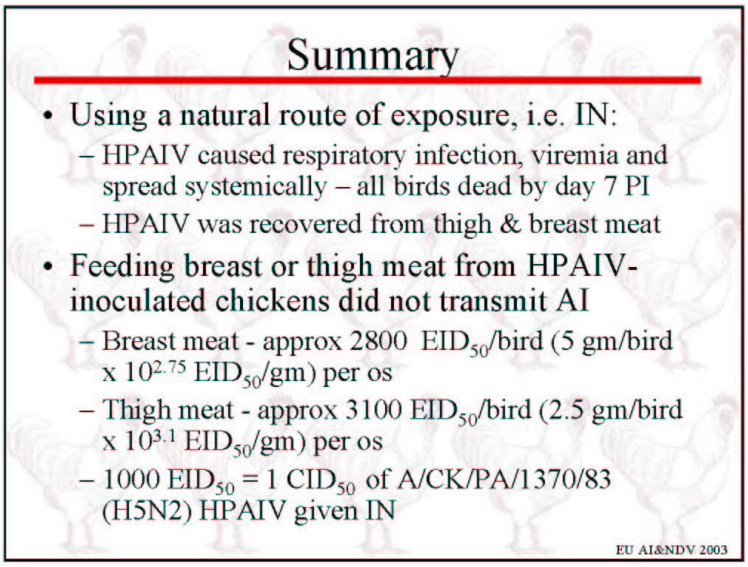
7


Summary
• Using a natural route of exposure, i.e. IN:
– HPAIV caused respiratory infection, viremia and spread systemically – all birds dead by day 7 PI
– HPAIV was recovered from thigh & breast meat
• Feeding breast or thigh meat from HPAIV-inoculated chickens did not transmit AI
– Breast meat - approx 2800 EID_{50}/bird (5 gm/bird x $10^{2.75}$ EID_{50}/gm) per os
– Thigh meat - approx 3100 EID_{50}/bird (2.5 gm/bird x $10^{3.1}$ EID_{50}/gm) per os
– 1000 EID_{50} = 1 CID_{50} of A/CK/PA/1370/83 (H5N2) HPAIV given IN
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8

Surveys for avian influenza in poultry and wild birds in member states 2003

Ian Brown
Community Reference Laboratory
Veterinary Laboratories Agency, UK
December 2003

Background

- Control of highly pathogenic avian influenza (AI) (Directive 92/40/EEC)
- Surveillance not forseen in directive
- Low pathogenic strains not covered by directive may circulate and acquire virulence
- Severe economic losses may be alleviated by intervention strategies

Proposed AI definition changes

For the purpose of diagnostic procedures for the confirmation and differential diagnosis of avian influenza:

''Avian influenza' means an infection of birds caused by any influenza A virus which has an intravenous pathogenicity index in six-week-old chickens greater than 1.2 or any infection with influenza A viruses of H5 or H7 subtype.''

Programme objectives

• To investigate the prevalence of infections with influenza A viruses of H5 and H7 subtypes in different species of poultry as a precursor study for possible EU-wide monitoring

• 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

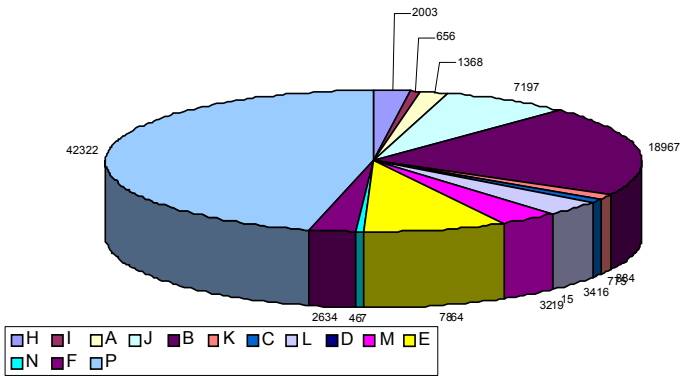
General structure of programme

- All categories of poultry
- Statistical based programme
- Sampling
  - adapted according to host
- Laboratory tests
- Wild bird surveillance optional
- Reporting (October 2003)

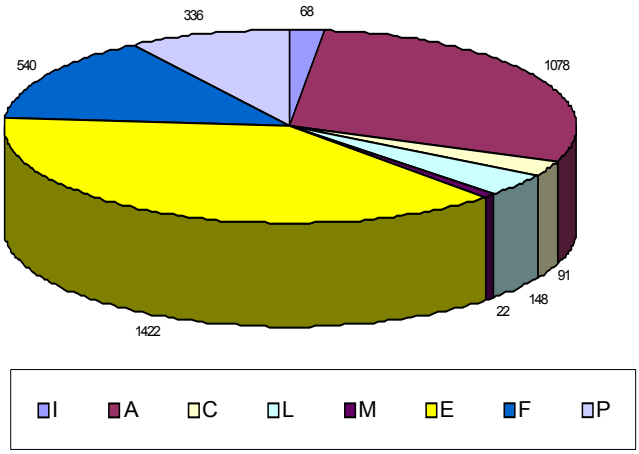
Data analysis problems

- Region definition
- Definition of holding
- Single sampling of individual holdings
- Data availability/accuracy for total number of holdings by class
- Variation in class definition

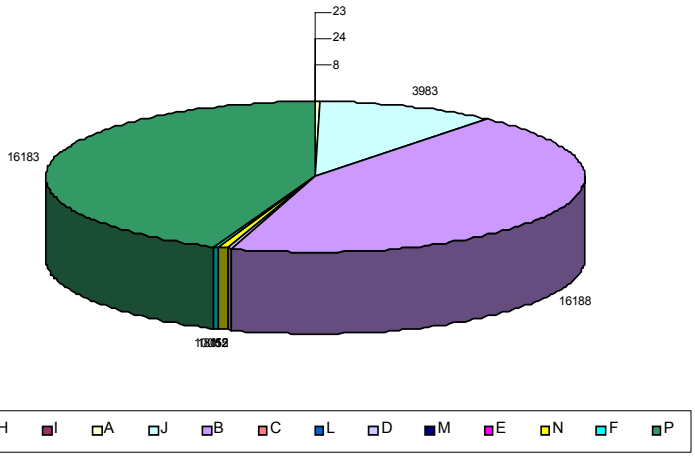
Total No. of Holdings of all Classes by Member State



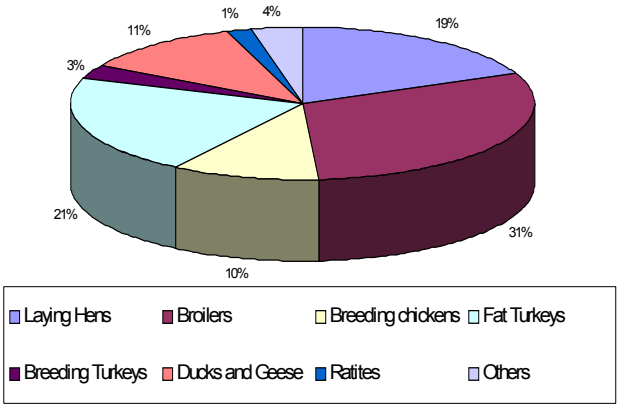
Total No. of Turkey Fattener Holdings



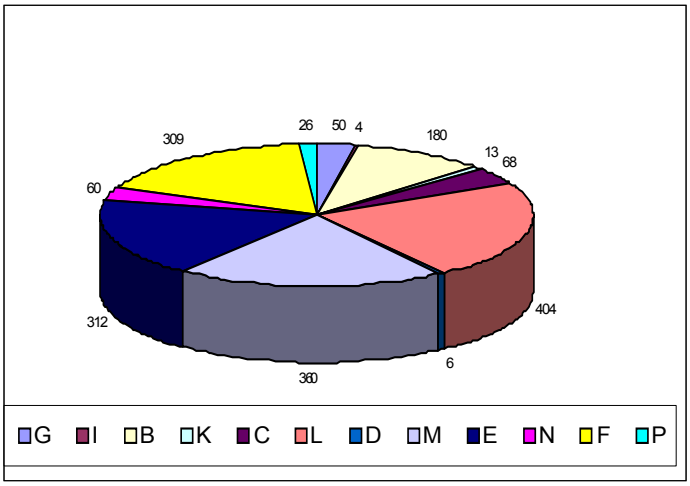
Total no. of Geese and Ducks Holdings



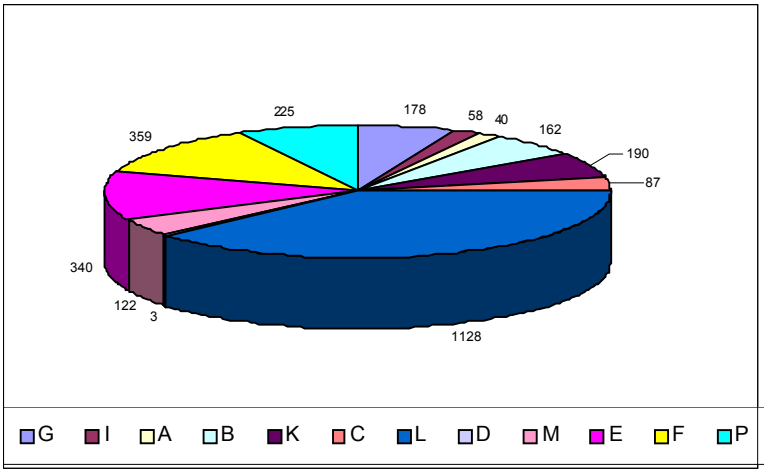
Percentage of classes sampled by holdings



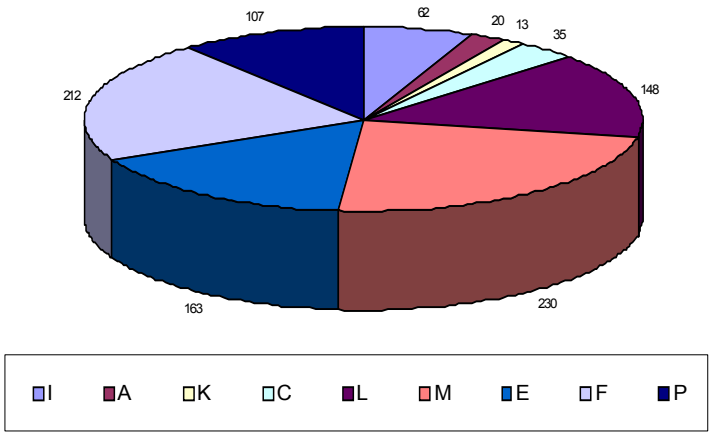
Total no. holdings of laying hens sampled



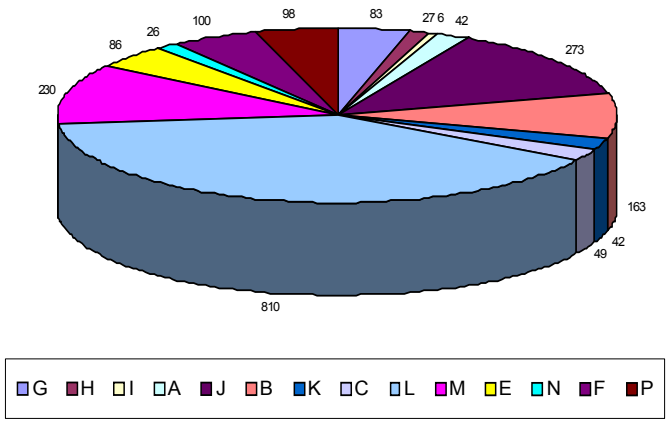
Total no. of broiler holdings sampled



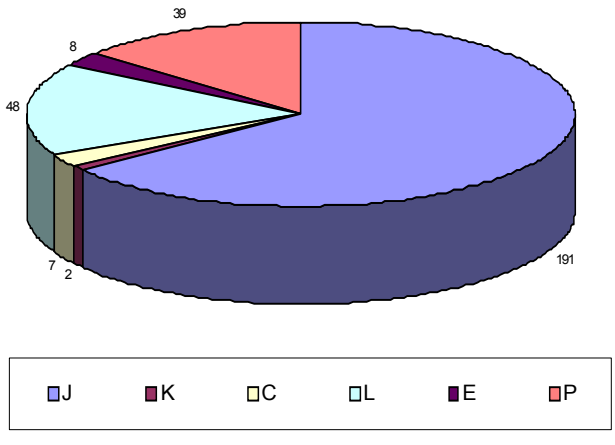
Total no. of chicken breeder holdings sampled



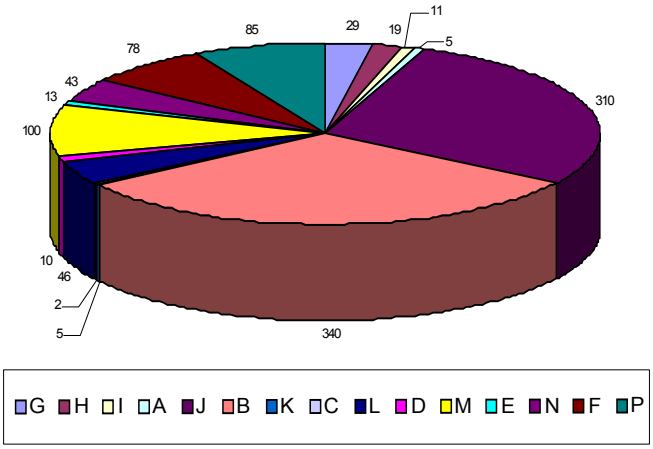
Total no. of turkey fattener holdings sampled



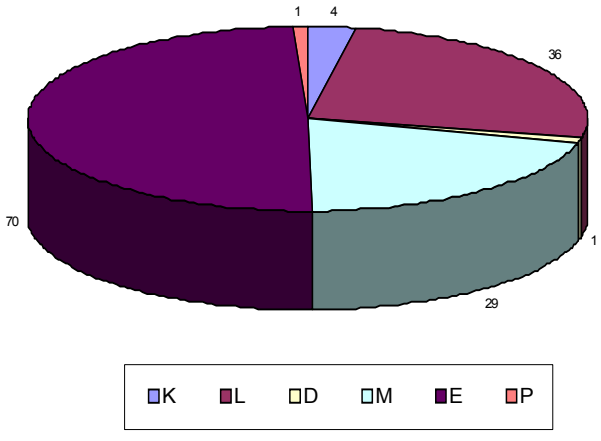
Total no. of turkey breeder holdings sampled



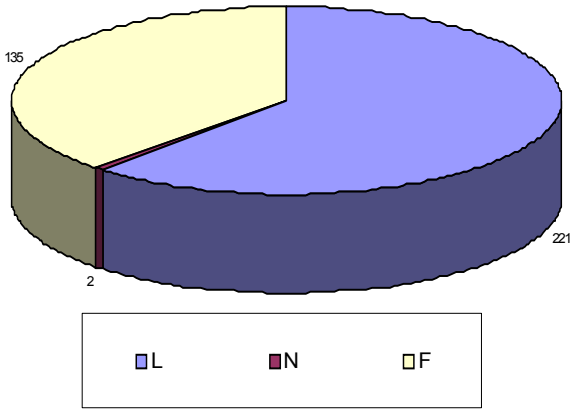
Total no. of duck and geese holdings sampled



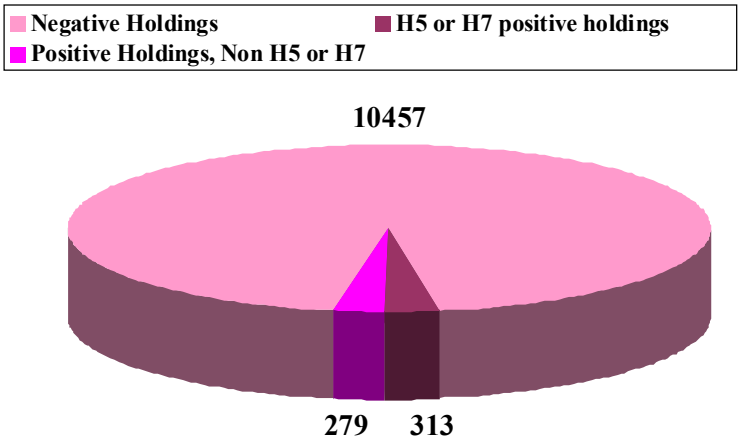
Total no. of ratite holdings sampled



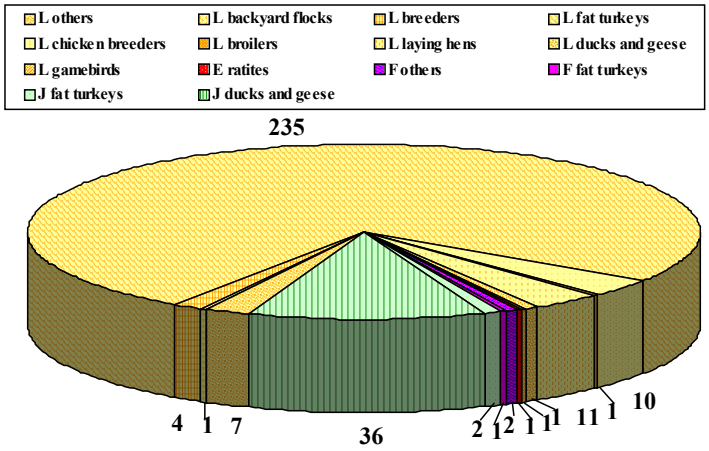
Total no. of other holdings sampled



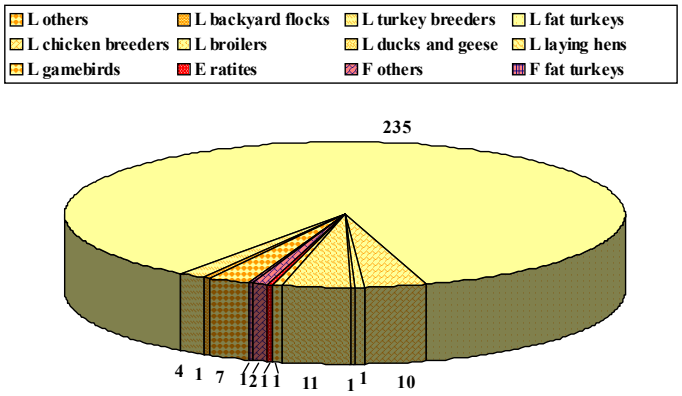
Results of total holdings tested



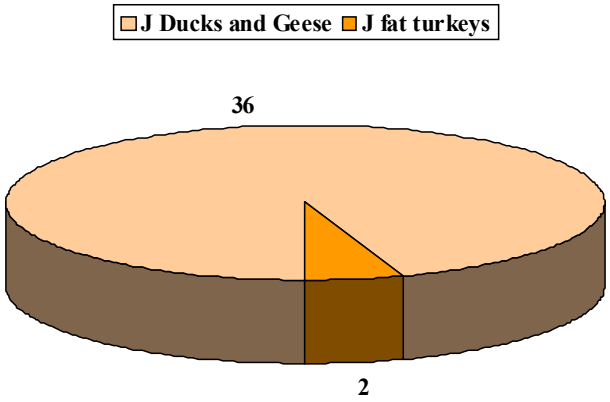
H7 and H5 positive holdings by MS and category



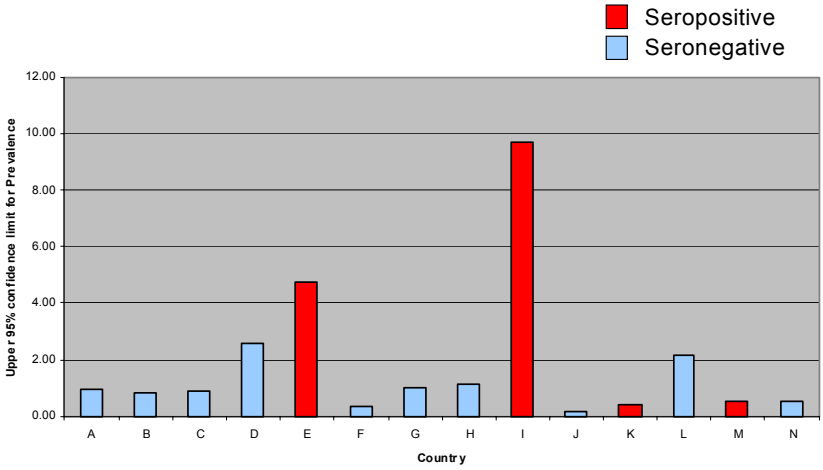
H7 positive holdings by MS



H5 positive holdings by MS



Upper 95% confidence limit for prevalence



Conclusions

- Prevalence of H7 and H5 viruses was reported in four MS's
- Two of these MS's had concurrent outbreaks with H7 in the wider/within region at the time of the survey
  - Infection in a number of hosts but principally fattening turkeys
- Very low prevalence to H7 in one other MS
- H5 prevalence in one MS, mainly in ducks
- H3N8 & H6N1 isolated from commercial ducks
- Overall the point prevalence study indicated low prevalence with estimates of limits for all MS's in the range 0.19 to 3.02%

AI survey in wild birds

- Diversity of species
  - waterfowl, shorebirds & other free-living birds
- Virus detection using faecal material
  - test sample pools from same host species
- Results received from 11 MS's
- 3777 samples examined
- Nine (0.002% isolation rate!) influenza A viruses
  - H10N5, Mallard
  - H7 x6 Ducks
  - H9N9 Shorebird (Knot)
  - H13N6 Jackdaw

Thank you for submitting results promptly

We look forward to receiving viruses isolated

Future

- Repeat of 2002/3 programme envisaged in 2004
- Guidelines will be reviewed
- Issues – questionnaire
  - Risk based
  - Regions/categories
  - Laboratory approaches
  - Information consistency

PREPARATION AND IMPLEMENTATION OF THE SIMULATION EXERCISES FOR THE CONTROL OF AVIAN INFLUENZA AND NEWCASTLE DISEASE

Jorgen M. Westergaard and Maria Pittman

INTRODUCTION

The legislation adopted in 1992 on Community measures for the control of avian influenza and Newcastle disease (1, 2) contains no specific reference to the implementation of simulation exercises, but in the annexes of the Directives 92/40/EEC and 92/66/EEC covering the criteria for contingency plans, it is stated: "training programs shall be established to maintain and develop skills in field and administrative procedures". When training aspects were discussed in the early 1990s it was understood that the conduct of simulation exercises should be a component of the established training programs.
The more recently adopted EU legislation on animal disease control measures has put emphasis on contingency planning with regard to OIE list A diseases and within this context it contains provisions for training of staff in the Member States responsible for the implementation of disease control and eradication measures. The legislation governing the control of classical swine fever (3) adopted in 2001 stipulates that "alarm drills" shall be conducted and the foot and mouth disease legislation (4) adopted in 2003 calls for "real-time alert exercises".
The aim of this paper is to highlight certain aspects related to the preparation and implementation of exercises simulating outbreaks of avian influenza (AI) or Newcastle disease (ND). For this purpose the following definitions shall apply.
"Simulation exercise" – means a model of a course of events related to one or more disease outbreaks where the participants of the exercise may know well in advance the date(s) the exercise shall take place, but not have advanced knowledge of the scenario prepared for the exercise.
"Real-alert exercise" – means a model of course of events related to one or more disease outbreaks where the participants of the exercise have no prior information about the time and the scenario prepared for the exercise.
"Disease eradication drill" or "Disease alarm drill" – means practice in dealing with a disease situation at a holding, at region or country level.

The main headings used in this paper are the following:

- Objectives
- Elements of simulation exercise
- Management of simulation exercise
- Preparation of scenario for a simulation exercise
- Database support
- Tasks to be performed during a simulation exercise
- Facilities for a simulation exercise

I. OBJECTIVES

The main objectives of a simulation exercise are to expose the participants to an AI or ND situation which requires:

- An analysis of a rapidly changing disease situation.
- Decision making on practical issues related to disease eradication measures such as depopulation of infected flocks, destruction of infected carcasses, cleansing and disinfection procedures and enforcement of movement restrictions.
- Operation of a disease control center and the coordination of disease prevention, control and eradication measures with a number of other official institutions and private organisations.

In addition to the above listed main objectives participants taking part in an exercise have the opportunity to:

- Assess the control measures given in the national legislation in force.
- Make use of contingency plans and operational manuals; this means being familiar with working procedures during outbreaks and making use of forms developed for disease notification, valuation and compensation of poultry flocks, dispatch of specimen for laboratory examinations, movement restrictions etc.

II. ELEMENTS OF A SIMULATION EXERCISE

1. The scope of the exercise shall be realistic. The scenario (problem) should be based on the characteristics of the poultry production in the country concerned and be of a sufficient magnitude to present a challenge to the participants.
2. The simulated "suspect" and "infected" holdings shall be holdings that actually exist. The geographical location shall be made available.
3. The control measures taken into account during the exercise shall include:
   - Disease investigation, including tracing (up-stream and down-stream) and identification of the index case
   - Collecting samples for laboratory examination
   - Stamping-out
   - Disposal of carcasses
   - Cleansing and disinfection
   - Establishment of movement restrictions
   - Pre-emptive slaughter
   - Emergency vaccination, where relevant
   - Restocking
   - Post-epidemic screening

4. The administrative measures taken into account during the exercise shall include:
   - Payments of compensation to farmers.
   - Recruitment of staff – payments, lodging, transport etc.
   - Procurement of equipment, disinfectants, protective clothing and vaccine
   - Financial aspects related to killing and disposal of poultry.
   - Preparation of legal texts related to movement restrictions and trade.
   - Providing information to national and international authorities and the press.

III. MANAGEMENT OF A SIMULATION EXERCISE

1. The Chief Veterinary Officer (CVO) should be in charge of the preparation and implementation of the exercise
2. The simulation exercise can be a kind of a classroom exercise. The demand for facilities, however, depends on the number of tasks to be performed during the exercise. If possible the classroom exercise should be supplemented by aspects of more realistic nature.
3. The contingency plan and the operational manual shall be available to participants during the exercise.
4. The exercise shall preferably cover the whole period of an epidemic and include the lifting of movement restrictions. Time may be compressed. Careful considerations, however, should be given to actual time intervals as if the conditions were real.
5. Reporting of the simulation exercise should be completed in the following manner:
    - All completed forms, epidemiological reports and drafted legal provisions should be assembled in a comprehensive report. This report should include the reports and forms completed by the national disease control center and the reports and forms completed by members of the local disease control centers.
    - The content of the report should reflect the true capability of the participants dealing with assignments.
    - Organisation charts of the national and local disease control centers should be included in the report.
    - Maps showing the entire exercise, including infected premises, tracing operations, pre-emptive slaughter of flocks, restriction zones, disinfection points, abattoirs, rendering plants, bird markets, etc shall if possible be in the report.
    - The estimated cost of the epidemic must be included in the exercise report.
    - A summary of the exercise and an evaluation should be prepared for the report. All personnel should participate in the preparation of this section.

IV. PREPARATION OF SCENARIO FOR A SIMULATION EXERCISE

A scenario for a simulation exercise can be prepared in several different ways. One model is described below and it is based on that the scenario writer creates a new epidemic. It can be recommended that initially the writer prepares a diagram showing a number of outbreaks (for example 20) and indicates the potential epidemiological links between the outbreaks. Each outbreak shall occur on a holding/establishment that actually exists. Furthermore the writer decides on the date of confirmation of each outbreak and prepares a diagram showing the temporal distribution of the outbreaks.
During the preparation of the scenario special consideration should be given to the following elements.

1. The primary outbreak

A "primary outbreak' can be defined as the initial outbreak of a transmissible disease occurring in an area previously free of this condition. Within the EU legislation (5) it has been

defined as an outbreak not epidemiologically linked with a previous outbreak in the same region of a Member State or the first outbreak in a different region of the same Member State. The scenario writer shall decide whether or not the primary outbreak should be the index case. Information to be presented in relation to the simulated primary outbreak should refer to:

- Name of owner and location of holding
- Poultry population at the holding
- Disease situation and treatments carried out on the holding prior to confirmation of disease
- Potential source of infection
- Potential spread of infection from the holding.

2. Characteristics of the agent

The spread of a pathogen through a poultry population will mainly depend on the physico-chemical characters of the agent, the mode of transmission, the infectivity and virulence of the agent. Knowledge of these characteristics together with knowledge of incubation periods, clinical signs and symptoms of disease must be taken into account, when secondary outbreaks are described.

3. Secondary outbreaks

With the aim of ensuring that participants of a simulation exercise are being exposed to a large number of different disease situations and to the application of different control and eradication methods, the scenario writer shall select holdings for secondary outbreaks which:

- Have different sizes of poultry population
- Have poultry of different species
- Have different types of production systems (breeding poultry, productive poultry, hatchery and back yard flocks)
- Are located in different environments (areas with low, medium or high density of susceptible animals; areas where wild fauna may have contact with susceptible poultry species)

Information should be available for each holding as shown in Annex 1.

4. Epidemiological links

Much information about epidemiological links between different outbreaks of AI and ND is available in the literature. The scenario writer can, by selecting the epidemiological links for the exercise, decide to what extent the simulated epidemic shall reflect a specific characteristic of the agent, a shortcoming in the implementation of certain disease control measures or a combination of both.

5. Temporal distribution of outbreaks

The scenario writer can by plotting the simulated outbreaks on a time scale, evaluate to what extent the temporal distribution of outbreaks can be considered realistic. In preparing the

graph or diagram consideration must be given to the characteristics of the agent, the host(s) and spread of the agent.

V. DATABASE SUPPORT

The simulated "infected" holdings must be holdings that actually exist. The scenario writer shall, during the preparation of an exercise, have access to up-dated databases or records with information on the location of holdings, the poultry population on the holdings and, if possible, movements of poultry to and from the holding during the previous 30 days.

VI. TASKS TO BE PERFORMED DURING A SIMULATION EXERCISE

During the preparation of a simulation exercise the scenario writer decides on the tasks to be performed by the participants during the exercise. The tasks shall reflect the disease situation and evolution of disease described in the scenario and the objectives of the training programme. It is foreseen that participants will be allocated to different working groups and that the number of participants in the different groups may vary; 4 – 6 participants per group is suggested. Each group should identify a group leader.
Information on the initial and changing disease situation described in the scenario can be presented to the participants/working groups:

- at a daily plenary session; usually in the morning
- through messages given with certain intervals (hours) to the individual working groups. The messages contain information on new developments and may be given via emails or in envelopes.

1. Tasks to be performed in relation to a primary AI or ND outbreak

The tasks to be performed will be based on the information provided in the scenario. In this paper it is suggested that the participants be allocated into 5 different working groups and engaged in the control of AI.

Group 1

Based on a serious **suspicion** of AI, the group should:

a) prepare the measures to be taken at the suspect holding including:

- measures preventing the spread of infection/disease from the holding
- inspection and clinical examination of poultry
- collection of appropriate number of high quality samples for laboratory examination
- transport of samples to the laboratory
- prepare a detailed plan (lay-out of the holding) showing all units on the holding. The activities carried out in each unit shall be identified and the history of disease in the different units shall be noted.
- make a count of all live poultry in the various categories on the holding

- make a list of birds sick and dead on the holding
- make an epidemiological enquiry

b) provide information to the Regional Veterinary Officer or the CVO on data necessary for disease notification.

Group 2

Following **confirmation** of AI, the group should make arrangements for:

a) overall measures required to prevent the spread of the disease from the infected holding
b) the report to be submitted to the CVO concerning the disease situation
c) valuation of poultry
d) killing of poultry
e) disposal of poultry, products and waste of poultry origin
f) completing the epidemiological enquiry
g) cleaning and disinfection of contaminated equipment and buildings

The tasks to be carried out by the groups 1 and 2 during the first day of the exercise should preferably to be carried out at the holding of the primary outbreak.

Group 3

Following **confirmation** of AI, the group should take actions to prevent further spread of the disease. These include:

a) the registration of all poultry flocks/holdings situated within a distance of 1 km, 3 km and 10 km of the infected farm. The number and species of poultry on each holding shall also be registered.
b) defining the surveillance program and the control of the movement of people and animals in the surrounding area (the 1000 meter-zone, the protection zone and the surveillance zone)
c) establishing the local crisis centre for disease control
d) announcements and publication for private practitioners, poultry producers and haulers in the region
e) possible control measures for the use of hatcheries, abattoirs and egg packing plants
f) evaluation of the needs for manpower and equipment to carry out the measures (a) – (e)

Group 4

Following **confirmation** of AI and based on the available information and documents the group should analyse the following:

a) All live poultry, hatching eggs and poultry products imported during the past 30 days

b) All live poultry, hatching eggs and poultry products exported to other countries during the past 30 days.
c) Movement of susceptible birds through markets during the past 30 days
d) List of products originating from poultry considered as safe for international trade.

The analysis should take into consideration the potential spread of disease by live poultry, hatching eggs and poultry products. Furthermore the group should estimate the volume of exported poultry and poultry products which may be returned from abroad.

Group 5

Following **confirmation** of AI the group shall prepare reports/informative notes to be submitted to:

a) OIE
b) EU
c) Ministry of Agriculture/Health
d) Private practitioners
e) The meat , egg and feed industries
f) The media.

The information should cover the main veterinary issues concerning the disease situation, control measures, vaccination options, potential developments and economic aspects. This group should include staff from the central veterinary administration.

2. Tasks to be performed in relation to secondary AI outbreaks

The exercise will be based on data in the scenario on secondary outbreaks.

Group 1

Based on the information on a number of secondary AI outbreaks, the group should determine:

a) overall measures required to prevent the spread of the disease from infected holdings
b) the report to be submitted to the CVO concerning the disease situation
c) valuation and killing of poultry
d) disposal of animals, products and waste of poultry origin
e) cleaning and disinfection of the infected premises
f) completing the epidemiological enquiry
g) control regime for holdings under observations

Group 2

Based on the confirmation of several AI outbreaks, the group should take actions to prevent further spread of the disease. These include:

a) Defining the surveillance program and the control of the movement of people and animals in the surrounding areas (protection and surveillance zones).
b) establishing local crisis centre(s) for disease control
c) announcements and publications for private practitioners, poultry producers and haulers in the region
d) possible control measures for the use of hatcheries, abattoirs and egg packing plants
e) assessment of vaccination against AI; preparation of local vaccination plan dealing with: vaccination strategy, area, poultry species, vaccine doses, timetable, staff and equipment required, legislation for compulsory vaccination, payment of costs.

Group 3

Based on several confirmed cases of AI the group should prepare further actions to be taken to prevent the spread of the disease concerning:

a) Products returned from other (third) countries
b) Collection and processing of products originating from poultry kept within the surveillance zone and assessment and collection of dead animals
c) Tasks in relation to animal welfare on farms situated in the surveillance zone/restricted area
d) Assessment of the capacity of abattoirs and rendering plants, recruitment of staff and other social aspects
e) Instructions or procedure concerning more general control measures (pet birds)

Group 4

Based on confirmed outbreaks of AI the group should submit information on costs concerning:

a) compensation for owners
b) killing and disposal of poultry
c) cleaning and disinfection of infected premises
d) operation and management of a crisis centre
e) actions to be carried out by other than for veterinary crisis services (Police, army, etc.)
f) implementation of vaccination plan; in case of vaccination
g) additional employment, equipment rental and compensations for possible damages or losses of income

Group 5

Based on the confirmation of AI outbreaks, the group should prepare the report based on the current situation for:

a) OIE
b) EU
c) Ministry of Agriculture/Health
d) Private practitioners

e) The meat, egg and feed industries.
f) The media.

The information should contain the main veterinary issues concerning the disease situation, control measures, vaccination situation, potential developments and economic aspects.
The group should furthermore ensure that

- a single file is prepared for each farm where AI has been recorded. The file should contain all relevant information on epidemiology, applied control measures and economic aspects
- an evaluation is made concerning the needs for manpower and equipment for implementation of eradication measures to continue for 50 outbreaks during the next 30 days.

3. Tasks to be performed in relation to secondary AI outbreaks and in the immediate post-epidemic period

The exercise will be based on information on secondary outbreaks and the fact that about 30 days have elapsed since the last outbreak was confirmed.

Group 1

The group shall describe the procedures for restocking the depopulated holdings. The description shall include:

a) a time table for restocking based on the provisions of the AI legislation
b) step by step plan of the actions to be taken in relation to restocking
c) procedures for purchase of poultry for restocking
d) surveillance and trade conditions for repopulated holdings

Group 2

The group shall prepare a program for post-epidemic screening for lifting movement restrictions. The program shall cover:

a) domestic and wild birds to be tested
b) Number and type of samples to be collected
c) Organisation of sampling in the field
d) Organisation of testing of samples at the National AI diagnostic laboratory
e) Timetable for implementation of the screening programme

Group 3

Based on the recorded outbreaks of AI during the simulation exercise, the group should submit information of costs concerning:

a) compensation for owners
b) killing and disposal of poultry
c) cleaning and disinfection of infected premises
d) operation and management of a crisis centre

e) actions carried out by other than the veterinary crisis services (Police, army, etc.)
f) laboratory costs; if applied, implementation of vaccination program
g) additional employment of staff, equipment rental and compensation for possible damage or loss of income

Group 4

The group shall prepare a diagram indicating the epidemiological links between all recorded outbreaks and a diagram showing the temporal distribution of outbreaks. Furthermore it shall prepare the lay-out of the report to be presented to the EU and other trading partners concerning the eradication of the disease.

Group 5

Based on the recorded outbreaks of AI, the group should prepare the report based on the current situation for:

a) OIE
b) EU
c) Ministry of Agriculture/Health
d) Private practitioners
e) The meat, egg and feed industries
f) The media.

VII. FACILITIES FOR SIMULATION EXERCISE

The facilities required for the implementation of a simulation exercise may vary. The main features of minimum and recommended facilities are given below.

Minimum facilities

A simulation exercise can be conducted as a paper exercise. The facilities required are a number of rooms with sufficient space for the working groups to perform their tasks and one room large enough for plenary sessions. The plenary sessions will in particular deal with information on changing disease situation, tasks to be performed by working groups, daily reporting by working groups, discussions and evaluation of work carried out. All rooms should have telephones and computers; the room for the plenary sessions should have audio-visual aid equipment.

Recommended facilities

A holding with breeding or production poultry should be available for the demonstration of activities to be "performed" in relation to a primary outbreak. These activities relate to: preventive measures at time of disease investigation; the disease investigation, collection and dispatch of samples to the laboratory, valuation of animals, depopulation and disposal of carcasses, epidemiological investigation, cleansing and disinfection.

Rooms for working groups and plenary sessions should be available as described above and preferably in the vicinity of the holding selected for the primary outbreak. Whenever possible the facilities of a disease control center should be used.

REFERENCES

1. Council Directive 92/40/EEC introducing Community measures for the control of avian influenza. OJ L 167, 22.06.92 p.1
2. Council Directive 92/66/EEC introducing Community measures for the control of Newcastle disease. OJ L 260, 05.09.92. p.1
3. Council Directive 2001/89/EC on Community measures for the control of classical swine fever. OJ L 316, 1.12.2001, p. 5.
4. Council Directive 2003/85/EC on Community measures for the control of foot-and-mouth disease amending Directive 92/46/EEC. OJ L 306, 22.11.2003, p.5
5. Council Directive 82/894/EEC on notification of animal diseases within the Community. OJ L 378, 31.12.1982. p. 58.

Annex 1

Secondary outbreaks, Fact sheet on "infected" holdings

For the purpose of a simulation exercise within the context of contingency planning a fact sheet should be prepared on each holding. The fact sheet should give information on the following:

1. Name and location of holding
2. Size of flock (breakdown of flock)
3. Disease situation on the day of suspicion
4. Name of local veterinarian (private practitioner)
5. Movement of poultry to and from the holding during the last 30 days
6. Daily production and sale of eggs in case of layer flocks
7. Information on water and feed supply
8. Approximate amount of feed on the farm on the day of disease suspicion
9. Area (approximate number of square meters) of buildings used for keeping poultry
10. Area (approximate number of square meters) used for storage of feed and for manure.

COUNTRY REPORTS ON AVIAN INFLUENZA FOR 2002 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 30 questionnaires was sent to different laboratories. Responses were received for 13 laboratories of EU countries: Italy, Portugal, Spain, Greece, Ireland, UK, Denmark, Finland, France, The Netherlands, Sweden, Germany and Belgium, and 12 from Accession states or non-EU countries: Slovenia, Norway, Cyprus, Estonia, Bulgaria, Poland, Slovak Republic, Lithuania, Czech Republic, Turkey, Romania and Switzerland. The samples tested and the results for avian influenza are summarised in the following pages.

VIRUS ISOLATION REPORTS BY COUNTRY

BELGIUM/LUXEMBOURG

Samples tested

Type of bird	Sample	Method	Number
broilers	tissue samples	in eggs	81
	tissue samples	in cell culture	81
layers	tissue samples	in eggs	43
	tissue samples	in cell culture	43
pigeons	tissue samples	in eggs	38
	tissue samples	in cell culture	38
birds	cloacal swabs	in eggs	400

Influenza viruses isolated
None.

BULGARIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Method	Number
broilers	cloacal swabs	in eggs	34
pigeons	cloacal swabs	in eggs	16

Influenza viruses isolated
None.

Serological monitoring for avian influenza antibodies

Type of bird	Number of samples	Method	Result
broilers	435	ELISA	negative
ducks	150	ELISA	negative
turkeys	30	ELISA	negative
geese	25	ELISA	negative
pigeons	22	ELISA	negative
pheasants	52	ELISA	negative
quails	20	ELISA	negative

CYPRUS

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
ostriches	cloacal swabs	101
	tracheal swabs	114
	tissues	198
caged birds	tissues	15

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

Type of bird	Number of samples	Method	Result
chickens	394	AGID	negative
ducks	20	AGID	negative
turkeys	10	AGID	negative
ostriches	171	AGID	negative

CZECH REPUBLIC

Samples tested by inoculation into eggs

Birds	Sample	Number
broilers	tissues	22
pheasant	tissues	2
pigeons	tissues	4

Influenza viruses isolated

None

DENMARK

Samples tested by inoculation into eggs

Type of bird	Sample	Number
broilers	cloacal swabs	4470
	tracheal swabs	4470
fowl	tissues	3428
caged birds	tissues	652
ducks and geese	cloacal swabs	90
	tracheal swabs	90
	tissues	580
pheasants and partridges	tissues	504
turkeys	cloacal swabs	30
	tracheal swabs	30
	tissues	112
pigeons	tissues	88
ostriches	tissues	12
wild birds	faeces	900

Influenza viruses isolated.

Domestic ducks: 1 x H4N6, 1 x H6N8, 1 x H1N?.
Wild birds: 1 x H2N?, 1 x H13N?, 1 x H?N?.

ESTONIA

Samples tested

None

FINLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	5
turkeys	tissues	20
ducks	tissues	1
geese	tissues	3
layers	tissues	12

Influenza viruses isolated
None.

FRANCE

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
domestic fowl	tissues cloacal swabs	20 5
turkeys	tissues cloacal swabs	4 21
ducks (mule)	cloacal swabs	9
pigeons	tissues cloacal swabs	25 6
guinea fowl	tissues	2
goose	tissues	1
pheasant	tissues	3
pet birds including quarantine	tissues faeces	14 4
sentinel chickens	tissues cloacal swabs	8 1
great comorants	cloacal swabs	4
mallards (surveillance)	cloacal swabs	78
sentinel Pekin ducks	cloacal swabs	40

Influenza viruses isolated

Bird	***subtype***	***IVPI***	***HA0 cleavage site***	***conclusion***
turkey breeders	H6 (N5?)	< 0.3*	nd	LPAI
mule duck	H5 (N3?)	0.0	PQRETR↓GLF	LPAI
mallard (x2)	H1N1	0.0-0.1	nd	LPAI
sentinel duck (x2)	H9N2	0.0	nd	LPAI

* bacterial contamination interfering in increasing signs

GERMANY

Virus isolation

Samples tested

Type of bird	Sample	Method	Number
chickens	tissues	eggs	432
		cell cultures	289
turkeys	tissues	eggs	43
		cell cultures	53
ducks	tissues	eggs	29
		cell cultures	17
	cloacal swabs	eggs	160
geese	tissues	eggs	20
		cell cultures	9
	cloacal swabs	eggs	310
backyard poultry, ornamental chickens	tissues	eggs	212
		cell cultures	206
pigeons	tissues	eggs	526
		cell cultures	517
psittacine birds	tissues	eggs	205
		cell cultures	228
pet birds, zoo birds	tissues	eggs	574
	tissues	cell cultures	414
	cloacal swabs	cell cultures	398
small wild birds	tissues	eggs	50
		cell cultures	24
wild aquatic birds	pharyngeal and cloacal swabs	eggs	289

Influenza viruses isolated

1 x H3N2 from imported pet bird in quarantine
2 x H3N8 from imported pet birds in quarantine
1 x H3N8 from wild bird (*Anas crecca*), IVPI 0.0
3 x H4N6 from wild birds (*Anas crecca, Anas querquedula*), IVPI 0.0
19 x H6N2 from turkeys, IVPI 0.0

Serological monitoring for avian influenza antibodies

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

Type of bird	Number of samples	positive	Subtype
chickens	3,868	0	-
meat turkeys	18,850	396	H6

GREECE

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	47
	cloacal swabs	104
broiler breeders	cloacal swabs	31
layers	tissues	20
	cloacal swabs	125

Influenza viruses isolated
None

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
broilers imported from Italy	2272	AGID	negative
layers, breeders and turkeys	668	AGID	negative

IRELAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	tissues	24
turkeys	tissues	36
	cloacal swabs	69
	tracheal swabs	40
pheasants	tissues	14
	cloacal swabs	5
geese	tissues	3
	cloacal swabs	30
	tracheal swabs	30
starling	tissues	3
dove	tissues	1
pigeon	tissues	12
	cloacal swabs	1
swans	tissues	2
not stated	tissues	6

Influenza viruses isolated
None.

ITALY

Samples tested in eggs:

Type of bird	Sample	Number
broiler breeders	tracheal swabs	417
layer breeders	tissues pools of cloacal swabs	2 4
broilers	tissues tracheal swabs	1 11
layers	tracheal swabs cloacal swabs	11 3
rural chickens	tissues	1
turkey breeders	tissues tracheal swabs	2 13
meat turkeys	tissues tracheal swabs	19 148
pheasants	cloacal swabs tracheal swabs	1 8
guinea fowl	tracheal swabs	1
ostriches	tissues	1
ducks	tissues cloacal swabs	3 42
pigeons	tissues pools of cloacal swabs	2 3
grey partridge	cloacal swabs tracheal swabs	3 2
cormorant	tissues	9
psittacine	cloacal swabs	3

Influenza viruses isolated

Meat turkeys	75 x H7N3 (62 from IZSVE + 13 isolated in other laboratory)
Turkey breeders	2 x H7N3 (from IZSVE)
Broilers	2 x H7N3 (from IZSVE)
Broiler breeders	1 x H7N3 (from IZSVE)
Layers	3 x H7N3 (from isolated in other laboratory)
Guinea fowl	1 x H7N3 (from IZSVE)
Ostriches	1 x H7N3 (isolated in other laboratory)
Ducks	1 x H9N2 (isolated in other laboratory)

Characterisation of viruses isolated in Italy

Birds	***Subtype***	***IVPI***	***HA0 cleavage site***	***Conclusion***
Meat turkey	H7N3	0.00	PEIPKGR*GLF	LPAI
Turkey breeders	H7N3	-	PEIPKGR*GLF	LPAI
Broiler	H7N3	-	PEIPKGR*GLF	LPAI
Broiler breeders	H7N3	-	PEIPKGR*GLF	LPAI
Layers	H7N3	-	PEIPKGR*GLF	LPAI
Duck	H9N2	-	not done	LPAI
Ostrich	H7N3	-	PEIPKGR*GLF	LPAI
Guinea fowl	H7N3	-	PEIPKGR*GLF	LPAI

LITHUANIA

Serological monitoring for avian influenza antibodies

Type of birds	**No. of samples**	**Method used**	**Result**
poultry [24 farms]	1200	ELISA	negative

THE NETHERLANDS

Samples tested by inoculation into eggs:

Type of bird	**Sample**	**Number**
chickens	tissues	32
caged birds	cloacal swabs or faeces	7209

Influenza viruses isolated

Bird	**subtype**	**IVPI**	**HA0 cleavage site**	**conclusion**
exotic	H3N?	n.d.	VPEKQTR.GL	LPAI

NORWAY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
caged birds	tissues	9

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

Type of bird	No. of flocks	No. of samples	Method	Result
chicken (imports)	11	330	HI (H5/H7)	negative
turkey (imports)	2	60	HI (H5/H7)	negative

POLAND

Samples tested

Type of birds	Sample	Method	Number
chickens	tissues	eggs	5
chickens	tissues	cell culture	2
broiler chickens	cloacal swabs	eggs	30*
broiler breeders	cloacal swabs	eggs	50*
layers	cloacal swabs	eggs	40*
meat turkeys	cloacal swabs	eggs	40*
turkey breeders	cloacal swabs	eggs	10*
geese breeders	cloacal swabs	eggs	60*
duck breeders	cloacal swabs	eggs	20*
wild birds	cloacal swabs/feaces	eggs	640

*samples collected during serological surveillance in 2001

Influenza viruses isolated from wild birds

Bird	subtype	IVPI	HA0 cleavage site	Conclusion
seagull	H5N?	0.00	PQRETR*GLF	LPAI
robin	H5N?	0.00	PQRETR*GLF	LPAI

PORTUGAL

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broiler	tissue	1
chickens	tissue	2
pigeons	tissues /faeces	10
psittacines in quarantine	pool faeces	43
psittacines in quarantine	tissues	55
wild ducks	cloacal swabs	36
psittacines	tissues	18
partridge	cloacal swabs	7
other birds	faeces	1

Influenza viruses isolated

None

SLOVAK REPUBLIC

Samples tested by inoculation into eggs or cell cultures

Type of bird	Sample	Number
chickens	cloacal swabs	6
turkeys	cloacal swabs	3
ducks	tissues	2
bean goose	tissues	2
free living birds	tissues	2
pigeons	cloacal swabs	6
peafowl	tissues	1
other pet birds	cloacal swabs	3
falcon	cloacal swabs	1

Influenza viruses isolated

None

Serological monitoring for avian influenza antibodies

Type of bird	No. of samples	Method	No. sera positive
layers	126	ELISA	15*
layers	15	AGP	2*
layers	2	HI	0
turkey	65	ELISA	29*
turkey	29	AGP	0
broiler	41	ELISA	2*
broiler	2	AGP	0
pigeon	5	ELISA	0
wild bird	8	ELISA	0
other pet bird	3	ELISA	0

*positives were not H5 or H7

SLOVENIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	2
broiler breeders	tissues	2
turkeys	tissues	2
back yard hen	tissues	1

Influenza viruses isolated
None

SPAIN

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
barn owl	cloacal swabs	3
buzzard	cloacal swabs	2
canary	cloacal swabs	468
cock	cloacal swabs	7
	tissues	1
eaglet	cloacal swabs	1
falcon	cloacal swabs	1
fish eagle	cloacal swabs	1
gannet	cloacal swabs	1
goshawk	cloacal swabs	1
kestrel	cloacal swabs	1
ostrich	cloacal swabs	120
owl	cloacal swabs	3

partridge eagle	cloacal swabs	1
psittacines and passerines	cloacal swabs tissues	2815 37
seagull	cloacal swabs	5
Spanish imperial eagle	cloacal swabs	5
tawny owl	cloacal swabs	1
vulture	cloacal swabs	3
white stork	cloacal swabs	2

Influenza viruses isolated
None.

SWEDEN

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broiler breeders	tissues	19
broilers	tissues cloacal swabs	6 3
backyard poultry	tissues cloacal swabs	1 3
turkeys	tissues	3
ducks	tissues	3
pigeons	tissues	3
wild birds	tissues cloacal swabs	27 1

Influenza viruses isolated

None

Serological monitoring for avian influenza antibodies

Type of poultry	Flocks tested	Sera examined	Flocks positive	Sera positive
imported broiler breeders*	8	790		
broiler breeders	93	5797		
imported layer breeders*	7	696		
layer breeders	36	1812		
broilers	2	110		
imported turkey breeders*	7	700		
turkey breeders	7	435		
backyard poultry	1	44	1	15
ostriches	6	38		
wild birds		42		1

*in isolation

SWITZERLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
laying hens	tissues	6
broilers	tissues	46
turkeys	tissues	2
pigeons	tissues	3
pet birds	tissues	1
pheasant	tissues	3
feral birds	tissues	1

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	25	250	1	1
laying hens	23	315	3	4

TURKEY

Serological monitoring for avian influenza antibodies [using AGID].

Type of poultry	Flocks tested	Sera examined	Flocks positive	Sera positive
broilers	243	14,580	0	0
breeders	5	300	0	0
turkeys	7	420	0	0
layers	47	2,820	0	0
others	10	600	0	0

ROMANIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	cloacal swabs tissues	267 48
pigeons	tissues	1

Influenza viruses isolated
None

UK - GREAT BRITAIN

Samples tested

Type of bird	Sample	Method	Number
chickens	tissues	eggs	599
	tissues	cell cultures	103
	cloacal swabs	eggs	13
	cloacal swabs	cell cultures	5
turkeys	tissues	eggs	31
	tissues	cell cultures	19
game birds	tissues	eggs	131
	tissues	cell cultures	33
pigeons	tissues	eggs	148
	tissues	cell cultures	99
	cloacal swabs	eggs	20
waterfowl	tissues	eggs	42
	tissues	cell cultures	53
caged birds	tissues	eggs	286
	tissues	cell cultures	94
	cloacal swabs	eggs	32
	cloacal swabs	cell cultures	10
ostriches	tissues	eggs	16
	tissues	cell cultures	9
raptors	cloacal swabs	eggs	33
other birds	tissues	eggs	19
	tissues	cell cultures	24

Influenza viruses isolated

None.

DISCUSSION

Of the 30 laboratories that were sent questionnaires a total of 25 laboratories responded, 13 EU laboratories and 12 from accession or non-EU countries. This represents a decrease of one EU laboratory but an increase of 2 non-EU laboratories over the returns for 2001 (Alexander & Manvell, 2003).

The amount of diagnostic and surveillance work for avian influenza varied enormously with country from no virus isolation attempts to considerable diagnosis in the face of outbreaks of LPAI infections in poultry and wide scale surveillance of domestic, quarantine and wild birds. The overall isolation attempts for avian influenza are summarised in Table 1. The overall total of 35,374 is more than 4 time the total of 8,498 in 2001.

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

Type of bird	Number countries reporting attempts	Number samples T = tissues/tracheal swabs C = cloacal swabs/faeces	Total samples
chickens	18 11	T 10,615 C 5,290	15,905
turkeys	10 5	T 577 C 173	750
ducks & geese	9 6	T 886 C 562	1,448
game birds	7 3	T 185 C 15	200
ostriches	4 1	T 350 C 120	470
pigeons	9 6	T 1,427 C 42	1,469
cage, zoo, pet, quarantine etc	8 7	T 1,938 C 11,030	12,968
other birds including wild birds	6 8	T 156 C 2,008	2,164
	TOTALS	**T 16,134** **C 19,240**	**35,374**

A total of 126 LPAI influenza viruses was isolated from six countries (Table 2). However, 86 of the viruses isolated were associated with the presence of H7N3 LPAI virus in Italy. Thirteen AI viruses were isolated from wild birds.

Reference

ALEXANDER, D.J. & MANVELL, R.J. (2003). Country reports on avian influenza and Newcastle disease for 2001 based on responses to the questionnaire. Proceedings of the Joint 8th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of countries of the European Union, Padova, 19-21 June 2002 pp 14-33.

Table 2 Summary of influenza viruses isolated by countries responding to the questionnaire

Country	Type of bird	Number	Subtype
Denmark	domestic ducks	1	H4N6
		1	H6N8
		1	H1N?
	wild birds	1	H2N?
		1	H13N?
		1	H?N?
France	turkey breeders	1	H6N5?
	mule duck	1	H5N3?
	mallard	2	H1N1
	sentinel ducks	2	H9N2
Germany	turkeys	19	H6N2
	quarantine caged birds	1	H3N2
		1	H3N8
	wild ducks	1	H3N8
		3	H4N6
Italy	chickens	6	H7N3
	turkeys	77	H7N3
	guinea fowl	1	H7N3
	ostriches	1	H7N3
	ducks	1	H9N2
The Netherlands	quarantine caged birds	1	H3N?
Poland	seagull	1	H5N?
	robin	1	H5N?

Avian Influenza Control Strategies in the United States of America

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AI Control Basics

- **Strategies for dealing with avian influenza are developed to achieve one of 3 goals or outcomes:**
  - *Prevention*: preventing introduction of AI
  - *Control*: reducing losses by minimizing negative economic impact through management practices
  - *Eradication*: total elimination of AI
- **These goals are achieved through various strategies developed using universal components:**
  - Biosecurity
  - Diagnostics and surveillance
  - Elimination of AI virus infected poultry
  - Decreasing host susceptibility to the virus
  - Education

EU AI&NDV 2003

I. H5 and H7 HPAI Control in USA

- Three HPAI outbreaks:
  - 1924-25, Northeast LPM, & supplying farms of Northeast & upper Midwest
  - 1929, few New Jersey farms
  - 1983-84, primarily PA, some of VA and MD
- Federal laws and regulations - USDA the authority to declare an animal health emergence
- Eradication programs can be conducted in cooperation with state governments
- USDA Emergency Response Plan (1998)

EU AI&NDV 2003

I. H5 and H7 HPAI Control in USA

Eradication Strategy

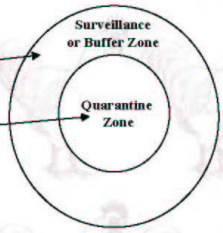
- Quarantine
- Stamping-out policy & carcass disposal
- C&D of equipment and farms
- Surveillance & diagnostics (1983-84)
- Indemnities paid (1983-84)
- Vaccination as an option (1998)

EU AI&NDV 2003

I. H5 and H7 HPAI Control in USA

Vaccination as a Tool During Eradication

- **Vaccine uses:**
  - **"Ring" vaccination**
  - **"Suppressor" vaccination**



- **Licensed vaccines:**
  - **H5 – full licensure: fowl poxvirus recombinant with H5 AI gene insert and inactivated whole H5 AI virus**
  - **H7 – conditional licensure: inactivated whole H7 AI virus**

EU AI&NDV 2003

II. H5 and H7 LPAI Control in USA

Control or Eradication Program (State Responsibility)

- Biosecurity
- Diagnostics and surveillance
- Elimination of infected birds (controlled marketing or depopulation); indemnities are inconsistent
- In some situations, vaccination
  - USDA-licensed: autogenous, conditional or full
  - Field use: since 1995 has required both Federal and State approval

EU AI&NDV 2003

IIa. H5/H7 LPAI – Minnesota Turkeys

- Since 1978, 108 outbreaks involving turkeys on 1097 infected farms – 20 outbreaks H5 or H7
  - Historically, waterfowl source to range turkeys (peak 5%)
  - 1997, most of range rearing ended
- Industry implemented an AI eradication strategy
  - Education (includes risk communication within the poultry industry)
  - Monitoring
  - Responsible response (includes controlled marketing and enhanced biosecurity practices)
  - Vaccination
- Outcome of eradication in less than six months
- Voluntary cooperative Industry-State Program

EU AI&NDV 2003

IIa. H5/H7 LPAI – Minnesota Turkeys

- No indemnities - relied on marketing of recovered turkeys as financial compensation for participation
  - Moving or marketing of turkeys during the acute phase of infection, i.e. period of high AI virus excretion, was reported as a high-risk activity associated with spreading of the virus to other farms
  - Infected flocks were required to sit on the farm under quarantine for 1-2 weeks before sending to processing
- Prior to 1995, H5 & H7 vaccine use did not require USDA approval

EU AI&NDV 2003

IIb. H7N3 LPAI – Utah Turkeys

- 26 April 1995 - H7N3 LPAI virus isolated from commercial meat turkeys in a single production company in the Sanpete Valley, Utah
- Control measures implemented included informing growers of the outbreak, enhanced biosecurity, controlled marketing of recovered flocks, and C&D of housing.
- H7N3 autogenous inactivated AI vaccine was used
  - 20 June 1995, uninfected 3-8 week-old turkeys, single dose
  - Within 6 weeks, 150 flocks were vaccinated
  - Sentinel birds did not seroconvert over the next 6 months.
  - No state or federal indemnities

EU AI&NDV 2003

IIc. H7N2 Live Poultry Market System

- 1994-2001, several attempts at eliminating H7N2 LPAI
  - Identifying markets with infected birds
  - Closure of infected markets
  - Eliminating infected birds
  - C&D
- April 2002, a federal-multiple state cooperative program
  - 123 retail markets simultaneously closed in 6 northeastern states
  - Owners sold down their bird inventories the day before closure and kill all remaining birds on the day of closure.
  - Compensated $3000 for 3 day closure plus $1000 supplement if also handled red meat livestock
  - Owner cleaned and disinfected
  - Task force inspected before repopulated w/AI free birds

EU AI&NDV 2003

IId. H7N2 Pennsylvania 1996-97

- H7N2 LPAI spilled-over from LPM system - 18 layer, 2 layer pullet, and 1 meat turkey commercial farms
- Control strategy: placement of quarantine, immediate depopulation with *on-farm* burial or delayed depopulation with landfill burial, C&D of premises, and surveillance.
- 21 farms voluntary depopulation w/o federal indemnity
- 2 layer farms:
  - Chickens recovered & continued to produce market eggs
  - Within 6 months, AIV recovered from both flocks
  - Ultimately, chickens depopulated & buried in a landfill
- Request to vaccinate not approved by USDA
- Partial indemnity was paid by the state government and from an industry indemnity pool.

EU AI&NDV 2003

IIe. H7N2 Pennsylvania 2001-2002

- H7N2 LPAI LPM AIV ⇒ 2 broiler breeders & 5 broiler
- Broiler farms did partial load-outs and sold birds to wholesalers for LPM distribution
- Broiler breeders euthanized in the houses with CO_2 gas and transported to landfill for disposal.
- Broiler flocks: 1 marketed, 1 euthanized on site w/landfill disposal, & 3 euthanized and composed within the houses
- No vaccine used
- No federal indemnity paid
- Quarantine and surveillance.

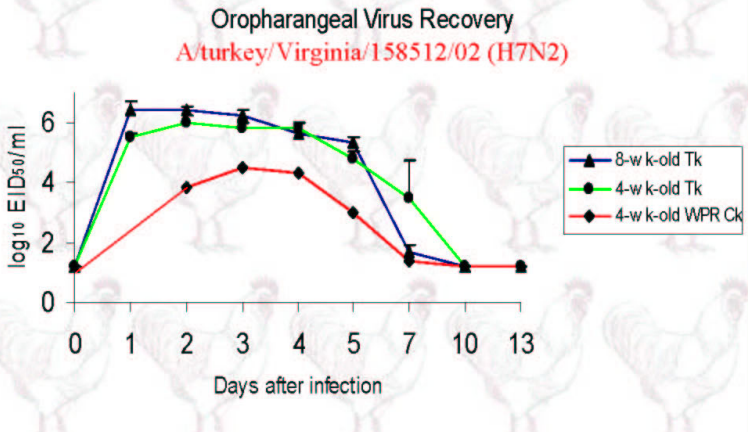
EU AI&NDV 2003

IIf. H7N2 Virginia 2002

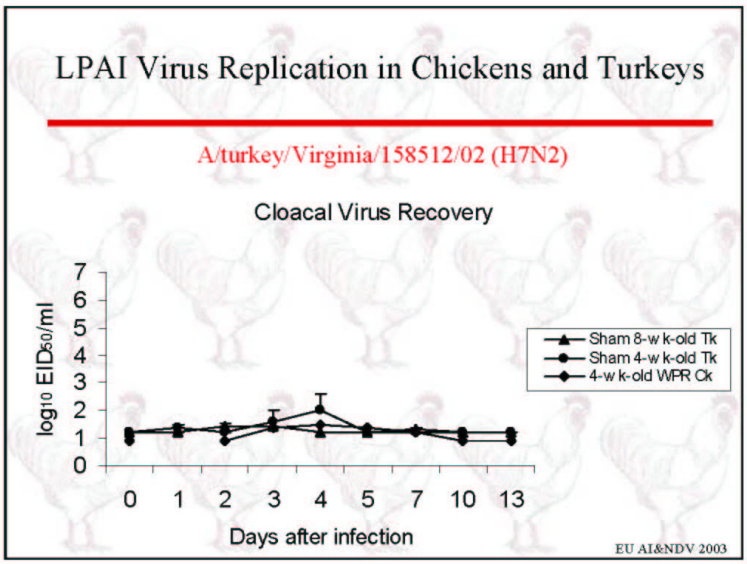
- 197 farms w/infected turkey and broiler breeders, egg layers, meat turkeys and broilers
- Eradication under USDA-Virginia-State Cooperative program funded by USDA
- Stamping-out and 100% indemnities
- Vaccine requested & approved by USDA for turkey breeders, but not approved by the state
- RRT-PCR validated & became primary test for AIV detection
- Cost: USDA - $81 million; $130 million losses

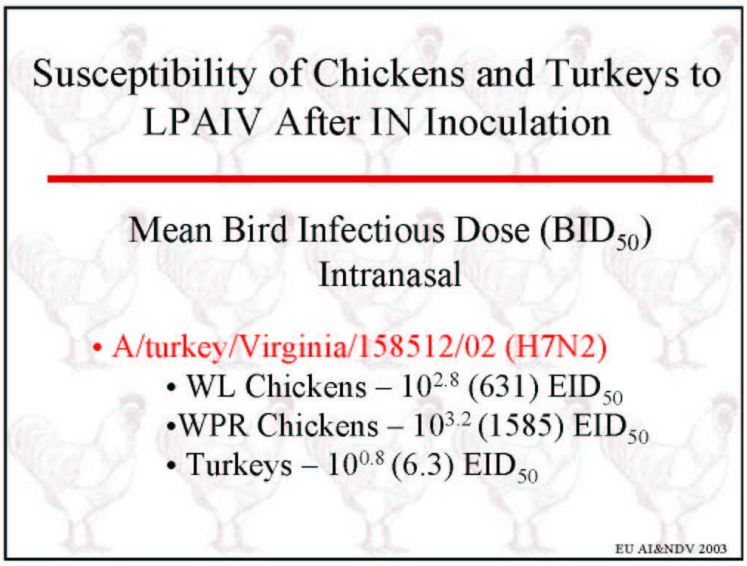
EU AI&NDV 2003

LPAI Virus Replication in Turkeys and Chickens



EU AI&NDV 2003


LPAI Virus Replication in Chickens and Turkeys
A/turkey/Virginia/158512/02 (H7N2)
Cloacal Virus Recovery
log10 EID50/ml
7
6
5
4
3
2
1
0
0 1 2 3 4 5 7 10 13
Days after infection
Sham 8-w k-old Tk
Sham 4-w k-old Tk
4-w k-old WPR Ck
EU AI&NDV 2003


Susceptibility of Chickens and Turkeys to LPAIV After IN Inoculation
Mean Bird Infectious Dose (BID_{50})
Intranasal
• A/turkey/Virginia/158512/02 (H7N2)
• WL Chickens – $10^{2.8}$ (631) EID_{50}
•WPR Chickens – $10^{3.2}$ (1585) EID_{50}
• Turkeys – $10^{0.8}$ (6.3) EID_{50}
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IIg. H7N2 LPAI – Connecticut

- One large layer company
  - Infected layers on 3 premises – 2.9 million birds
  - USDA requested the owner depopulate – no indemnities
  - State government and the company developed an alternative control strategy - prevent spread of the infection & eliminate infected birds
  - Basic strategy:
    - Isolate the farms (biosecurity practices)
    - Increase immunity in infected layers (single vaccination)
    - Replace infected layers with twice vaccinated pullets
    - Establish a monitoring program - unvaccinated sentinels (serology), screen daily mortalities (virus detection)
    - Develop a DIVA strategy using neuraminidase protein
  - 26 June 2003, last detection of H7N2 LPAI virus (RRT-PCR)
  - Continual evaluation of the control strategy

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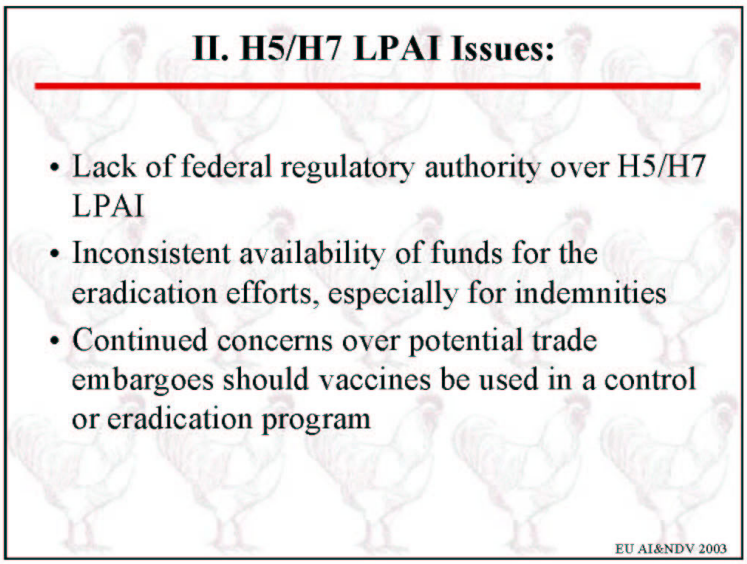
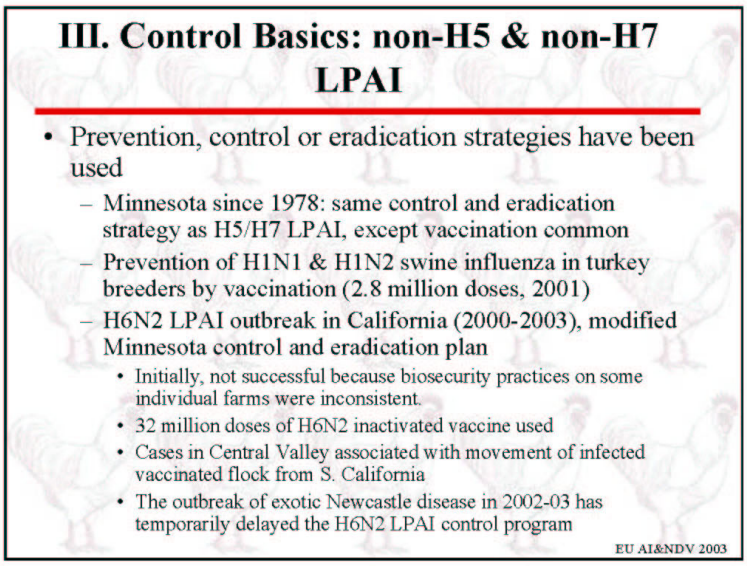
IIh. H7N2 LPAI – Rhode Island

- Single independent layer flock and farm – 32,000 mixed age layers, near Connecticut border
- 9 April 2003 - Visited by LPM dealer to purchase birds to sell followed by spike in mortality
- 16 April - RRT-PCR+
- Under state quarantine, no new birds allowed on farm
- No indemnity and no vaccination
- Allowed to sell washed, sanitized eggs
- Daily mortality taken to landfill
- Manure composted on farm
- At end of lay, farm will be depopulated, cleaned and disinfected

EU AI&NDV 2003


II. H5/H7 LPAI Issues:
• Lack of federal regulatory authority over H5/H7 LPAI
• Inconsistent availability of funds for the eradication efforts, especially for indemnities
• Continued concerns over potential trade embargoes should vaccines be used in a control or eradication program
EU AI&NDV 2003


III. Control Basics: non-H5 & non-H7 LPAI
• Prevention, control or eradication strategies have been used
– Minnesota since 1978: same control and eradication strategy as H5/H7 LPAI, except vaccination common
– Prevention of H1N1 & H1N2 swine influenza in turkey breeders by vaccination (2.8 million doses, 2001)
– H6N2 LPAI outbreak in California (2000-2003), modified Minnesota control and eradication plan
• Initially, not successful because biosecurity practices on some individual farms were inconsistent.
• 32 million doses of H6N2 inactivated vaccine used
• Cases in Central Valley associated with movement of infected vaccinated flock from S. California
• The outbreak of exotic Newcastle disease in 2002-03 has temporarily delayed the H6N2 LPAI control program
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IV. Proposed Control Plan for H5/H7 LPAI

- State-based and coordinated at Federal level
- Participation guarantees Federal assistance and indemnities
  - Indemnification not guaranteed but requested on an emergency basis
  - State surveillance plan & containment/control plan which is part of larger Federal-State-Industry initiatives
- Basic plan from USAHA, Committee on Transmissible Diseases of Poultry

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IVa. AI Vaccines

- Communicate to trading partners that vaccine is appropriate & should be available as part of a control strategy
- Establish a vaccine bank – 12M doses (2 H5's and 2 H7's, 3M doses each)
- Fund AI vaccine research – ARS
- Fund NVSL for diagnostics and serological test reagents

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IVb. Surveillance for Commercial Poultry National Poultry Improvement Plan (NPIP)

- Official State Agencies will administer the plan
- Participants:
  - Table-egg layers
  - Meat-type chickens
  - Meat-type turkeys
- Designation: "U.S. H5/H7 Avian Influenza Monitored"
- Testing: AGID and ELISA
  - AGID testing of all ELISA+

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Surveillance (NPIP): Table-Egg Layers

- Participants: flocks >75,000
- State designation as monitored when all table-egg flocks in state are monitored
- Surveillance:
  - 30 birds or 30 eggs during a 12 month period within 2 weeks of depopulation
  - AGID+ sent NVSL for subtyping
  - Flocks found to be infected should be quarantined by Official State Agency

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Surveillance (NPIP): Meat-Type Chickens

- Participants: Federally-inspected meat-type chicken slaughter plants with >200,000/wk
- State designation as monitored when all meat-type chicken slaughter plants are monitored
- Surveillance:
  - Minimum of 10 birds per shift test negative at slaughter or within 5 days of slaughter
  - AGID+ sent NVSL for subtyping
  - Flocks found to be infected should be quarantined by Official State Agency

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Surveillance (NPIP): Meat-Type Turkeys

- Participants: Federally-inspected meat-type turkey slaughter plants with >2 million/yr
- State designation as monitored when all meat-type turkey slaughter plants are monitored
- Surveillance:
  - Minimum of 60 birds per month test negative at slaughter. Recommended from turkeys over 10 wks of age with respiratory signs, depression or decreases in food and/or water intake
  - AGID+ sent NVSL for subtyping
  - Flocks found to be infected should be quarantined by Official State Agency

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Surveillance (NPIP): Breeding Flocks

- Additional current monitoring: Egg-Type Chicken Breeders, Meat-Type Chicken Breeders and Meat-Type Turkey Breeders
- Passive (diagnostic) surveillance program
  - AI reportable to State Veterinarian
  - Examine all submitted cases of respiratory disease, unexplained drops in egg production and unexplained severe mortality for AI by serology and antigen detection – on commercial and non-commercial poultry and other birds

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Initial Containment and Control Program

- Emergence management committee appointed in advance – industry, laboratory & state agricultural individuals
- Written minimum biosecurity and emergence disease awareness program in place
- Detailed procedures for initial handling and investigation of suspicious cases
- Initial strict quarantine of all presumptive and confirmed index cases

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Initial Containment and Control Program

- Immediate construction of geographically appropriate infected and control/monitoring zones, conduct epidemiological surveys for contacts and details of the movement and other disease control measures taken
- Details of the nature of the increased monitoring activity
- Detail plans for disposal of infected flocks
- Detailed plans for cleaning, disinfection and down time & plans for repopulation, & quarantine & monitoring of repopulated flocks

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V. Concluding Thoughts

- Need acceptance of the idea that control methods besides stamping-out can be effect and less costly for eradicating H5 and H7 LPAI. LP and HPAI have different pathogenesis of infection, different virus shed rates and different rates of transmission, thus their risks are different
- Acceptance of the compartmentalization concept is critical for developing new control and eradication methods for HPAI and for including H5 and H7 LPAI as "notifiable AI"

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V. Concluding Thoughts

- Federal-state cooperative control and eradication programs need to be developed with financial incentives for rapid detection and elimination of index cases of H5 and H7 LPAI
- USDA needs legal authority to control H5 and H7 LPAI including financial resources to pay indemnities

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Disease Control

Vaccination can only be a single tool in multiple-procedure strategy

But it is a tool we should use!!!

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Proceedings of the 5th International Symposium on Avian Influenza

- Available in September 2003
- Avian Diseases (Special Issue) 47:783-1268, 2003
- CD-ROM: Proceedings of the 1st-4th symposia

$50 - American Association of Avian Pathologists (953 College Station Rd, Athens, Georgia 30602-4875, tel: 706-542-5645, Fax: 706-542-0249; AAAP@uga.edu)

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6th International Symposium on Avian Influenza

- **St. John's College, Cambridge, United Kingdom**
- **April 3-6, 2006**
- **Co-chairs: Ilaria Capua (Italy) and David E. Swayne (USA).**

EU AI&NDV 2003


Thank you for your attention!
EU AI&NDV 2003

Annual Meeting of the National Laboratories for Newcastle Disease

TECHNICAL REPORT OF THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2002

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 and phylogenetic analysis

   c) Antigenic grouping of viruses

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**
401	188	113	154	199	294	385

1995	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**
605	284	227	285	357	704	316	333

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: *69 %*

WORK DONE: The 333 viruses submitted in 2002 were characterised as shown in Table 2.

Table 2: Identification of viruses submitted to the CRL in 2002

Virus identification	Number
Influenza A viruses	**137**
Paramyxoviruses	**178**
APMV-1 [NDV]	152
APMV-2	9
APMV-3	15
APMV-7	2
others	***18***
reovirus	2
poxvirus	2
untyped	2
virus not viable	12

In addition to identification, 17 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 a number 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: ***65%***

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: *14 %*

WORK DONE: The 152 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:

235 x 1.0ml ampoules of Newcastle disease (ND) antigen and 24 x 0.5ml ampoules of ND antiserum were supplied.

11 x 1.0ml of APMV-2, 13 x 1.0ml of APMV-3, 4 x 1.0ml of APMV-4, 4 x 1.0ml of APMV-6, 4 x 1.0ml of APMV-7, 4 x 1.0ml of APMV-8 and 4 x 1.0ml of APMV-9 antigen, and 14 x 0.5ml of APMV-2, 17 x 0.5ml of APMV-3, 8 x 0.5ml of APMV-4, 2 x 0.5ml of APMV-5, 8 x

0.5ml of APMV-, 9 x 0.5ml of APMV-7, 6 x 0.5ml of APMV-8 and 6 x 0.5ml of APMV-9 antiserum were distributed.

Certain ND virus specific monoclonal antibodies were also supplied to different laboratories: mAb 85 10 x 0.5 ml, mAb 161 18 x 0.5 ml and mAb 7D4 20 x 0.5 ml.

Estimated actual % resources**:* ***15%

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	75	7	150	8
APMV-3	45	8	75	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.*

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: *4 %*

WORK DONE: Antigens and antisera were prepared and dispatched to EU National Laboratories and those of accession countries [total 29 laboratories]

Estimated actual % resources**:* ***6%

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: *2 %*

WORK DONE: Results were received, analysed and an oral presentation made at the Annual Meeting in 2002. 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: *2 %*

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**:* ***3%

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: *3 %*

WORK DONE: In collaboration with the Commission's and the host's representatives the Annual Meeting was organised and held in Padova, Italy in June 2002.

Estimated actual % resources**:* ***3%

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 delayed 2001 proceedings before 2002 Annual meeting. Receive and collate submissions of 2002 meeting.*

***% Resources**: 2 %*

WORK DONE: Proceedings of the 2001 meeting were produced, the Proceedings of the 2002 meeting are being edited, some submissions had not been received by the end of 2002.

***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 2002 relating to the work of CRL for ND

12. ALEXANDER, D.J. & MANVELL, R.J. (2002). Report of the European Union Reference Laboratories for avian influenza and Newcastle disease 2000. Proceedings of the Joint 7th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Uppsala, April 2001 pp 6-13
13. ALEXANDER, D.J. & MANVELL, R.J. (2002). Country reports on avian influenza and Newcastle disease for 2000 based on responses to the questionnaire. Proceedings of the Joint 7th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of countries of the European Union, Uppsala, April 2001 pp 22-49
14. ALEXANDER, D.J. & MANVELL, R.J. (2002). Comparative tests for antigen identification in different National Laboratories 1999. Proceedings of the Joint 7th Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Uppsala, April 2001 pp 50-55
15. ALEXANDER, D.J. (2002). 75 years of Newcastle disease. Abstracts of Merial EU Technical Symposium 75 years of Newcastle disease, Tunisia September 2002.
16. STANISLAWEK, W.L., WILKS, C.R., MEERS,J., HORNER, G.W., ALEXANDER, D.J., MANVELL, R.J, KATTENBELT, J.A. & GOULD, A.R. (2002). Avian paramyxoviruses and influenza viruses isolated from mallard ducks (*Anas platyrhyncos*) in New Zealand. Archives of Virology 147, 1287-1302.
17. LI, Y., COLLINS, M.S., WHITELAM, G.C. & ALEXANDER, D.J. (2002). Rapid pathotyping of Newcastle disease virus using a single-chain Fv displayed on phage against the C-terminal end of the F2 polypeptide. Archives of Virology 147, 2025-2035.

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

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

COUNTRY REPORTS ON NEWCASTLE DISEASE AND OTHER APMV INFECTIONS FOR 2002 BASED ON RESPONSES TO THE QUESTIONNAIRE

Dennis J. Alexander and Ruth J. Manvell

Community Reference Laboratory for Newcastle disease
Veterinary Laboratories Agency Weybridge, New Haw, Addlestone,
Surrey KT15 3NB, United Kingdom.

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 30 questionnaires was sent to different laboratories. Responses were received for 13 laboratories of EU countries: Italy, Portugal, Spain, Greece, Ireland, UK, Denmark, Finland, France, The Netherlands, Sweden, Germany and Belgium, and 12 from non-EU countries: Slovenia, Norway, Cyprus, Estonia, Bulgaria, Poland, Slovak Republic, Lithuania, Czech Republic, Turkey, Romania and Switzerland. 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 ISOLATION REPORTS BY COUNTRY

BELGIUM/LUXEMBOURG

Bird	***Number***	***ICPI***	***amino acids***	***mAb group***	***conclusion***
pigeon	7	0.3-1.2	^{112}RRQKRF^{117}	P	PPMV-1
cage birds	1		^{112}RRQKRF^{117}	P	PPMV-1
cage birds	1				APMV-3

BULGARIA

No isolates

CYPRUS

No isolates

CZECH REPUBLIC

No isolates

DENMARK

APMVs

Fowl	3 x APMV-1
Ducks	3 x APMV-1
	1 x APMV-4
Partridges	1 x APMV-1
Pheasants	1 x APMV-1
	1 x APMV-2
Wild birds	2 x APMV-1
	1 x APMV-4
	1 x APMV-9
Caged birds	3 x APMV-2
	2 x APMV-3

APMV-1s

Bird	***ICPI***	***Amino acids***	***mAb group***	***conclusion***
Table-egg-layers	1.8	^{112}RRQRRF^{117}	C1	Newcastle disease
Table-egg-layers	1.7	^{112}RRQRRF^{117}	C1	Newcastle disease
Table-egg-layers	1.8	^{112}RRQRRF^{117}	C1	Newcastle disease
Duck	0.1	^{112}ERQERL^{117}	H	lentogenic APMV-1
Partridge	0.1	^{112}ERQERL^{117}	H	lentogenic APMV-1
Pheasant	0.2	^{112}GKQGRL^{117}	C2	lentogenic APMV-1
Goose (wild)	ND	^{112}GKQGRL^{117}	G/Q	lentogenic APMV-1
Goose (wild)	ND	^{112}GKQGRL^{117}	C2	lentogenic APMV-1
Mallard	0.0	^{112}ERQERL ^{117}	H	lentogenic APMV-1
Duck	0.2	^{11} ERQERL^{117}	H	lentogenic APMV-1

ESTONIA

No isolates.

FINLAND

No isolates

FRANCE

APMV isolates

Ornamental pigeon	4 x APMV-1 (PPMV-1)
Backyard chicken	1 x APMV-1
Mule ducks	1 x APMV-1
Wild mallard	1 x APMV-4

APMV-1s

Bird	*ICPI*	*Amino acids*	*mAb group*	*conclusion*
Pigeons* (x4)	0.9 to 1.3	^{112}RRQKRF^{117}	P	PPMV-1
Backyard chicken	0.0	^{112}GKQGRL^{117}	?**	avirulent
Mule ducks	0.3	^{112}GKQGRL^{117}	?**	avirulent

* ornamental ** no haemagglutination inhibition with mAb 7D4

GERMANY

APMV-1 isolates

Bird	*No.*	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
chickens	2	-[a]	-	E	lentogenic/vaccine
ornamental chicken	1	-	^{112}RRQKR*F^{117}	P	PPMV-1
goose	1	-	-	E	lentogenic/vaccine
pigeons	40 3	- -	^{112}RRQKR*F^{117} ^{112}GRQGR*L^{117}	P E	PPMV-1 lentogenic/accine
psittacines	2 1	- -	- -	E P	lentogenic/Vaccine PPMV-1
pet birds	7 1	- -	- -	P E	PPMV-1 lentogenic/Vaccine
wild aquatic birds	8	0.0	^{112}GRQGR*L^{117}	not done	lentogenic PMV-1

[a]not done

Other APMVs

Type of bird	*Number*	*Type*
ornamental chickens	2	APMV-3
psittacines	1 7	APMV-2 APMV-3
pet birds (in quarantine)	5 6	APMV-2 APMV-3

GREECE

No isolates

IRELAND

Bird	*ICPI*	*Amino acids*	*mAb group*	*conclusion*
pigeon	0.93	nd	P	PPMV-1
pigeon	1.21	nd	P	PPMV-1
pigeon	1.04	nd	P	PPMV-1

ITALY

broiler	5 x APMV-1 (2 isolated in other lab)
meat turkey	9 x APMV-1 (5 isolated in other lab)
pigeon	16 x PPMV-1 (12 isolated in other lab)
collared dove	16 x PPMV-1 (12 isolated in other lab)
duck	1 x APMV-1 (isolated in other lab)
guinea fowl	1 x APMV-1 (isolated in other lab)
psittacine	1 x A PMV-3 isolated in other lab)

APMV-1s

Bird	*ICPI*	*MAb group*	*Conclusion*
Broiler	0.1-0.2 0.6	E Not identifiable	4 x Vaccine 1 x Lentogenic
Duck	0.54	Not identifiable	1x Lentogenic
Pigeon	0.6 -1.1	P	11 x PPMV-1
Collared dove	0.6 -1.2	P	16 x PPMV-1
Guinea fowl	0.5	Not identifiable	1 x Lentogenic
Meat turkey	0.1-0.2 0.6	E Not identifiable	8 x Vaccine 1 x Lentogenic

LITHUANIA

No isolates

THE NETHERLANDS

Exotic birds in quarantine 7 x APMV-2

NORWAY

No isolates

POLAND

Bird	*ICPI*	*Amino acids*	*mAb group*	*conclusion*
free-living pigeon	0.42	^{112}RRQKRF^{117}	P	PPMV-1
pigeon	0.75	nd	P	PPMV-1
pigeon	1.04	^{112}RRQKRF^{117}	P	PPMV-1
mallard	nd	nd	nd	?

PORTUGAL

psittacines 1x APMV-3; 1x APMV-1

Characterisation of APMV-1 isolates

Bird	*ICPI*	*Amino acids at cleavage site*	*mAb group*	*Conclusion*
psittacine	1.70-1.85	GKQGRL RRQKRF	G	Mixture of virulent and avirulent virus

ROMANIA

No isolates.

SLOVAK REPUBLIC

Bird	*Number*	*ICPI*	*Conclusion*
pigeon	1	0.16	Low virulent PPMV-1

SLOVENIA

No isolates

SPAIN

No isolates.

SWEDEN

Bird	*Number*	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
broiler breeders	1	1.2	^{112}RRQKR∗F^{117}	P	PPMV-1

SWITZERLAND

pigeons 1 x APMV-1

Bird	*ICPI*	*amino acids*	*mAb group*	*conclusion*
pigeons	n.d.	n.d	P	PPMV-1

TURKEY

No isolates.

UNITED KINGDOM [GREAT BRITAIN]

Type of bird	*No isolates*	*Type of virus*
pigeons/doves	27	APMV-1
chickens	3	APMV-2
caged quarantine birds	1 3	APMV-2 APMV-3

APMV-1s

Bird	*Number*	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
pigeons	27	nd	nd	P	PPMV-1

Fourteen of the 25 laboratories reported no isolation of avian paramyxoviruses. The other 11 laboratories reported a total of 216 avian paramyxoviruses. One hundred and seventy-eight of these were APMV-1 viruses (Table 1). Forty-five of these APMV-1 viruses were of low virulence representing the isolation of live vaccine viruses or naturally occurring avirulent viruses. Three virulent viruses showing C1 monoclonal antibody binding were reported from chickens in Denmark and a virulent APMV-1 virus was obtained from a psittacine in Portugal. A total of 129 of the isolates was identified a APMV-1 viruses responsible for the

ongoing panzootic in pigeons [PPMV-1]. In all, 11 different laboratories reported PPMV-1 isolations and this emphasises the continued widespread presence of this virus in Europe and the continued threat this represents for domestic poultry and wild life.

Table 1 Summary of APMV virus isolations reported

Type of APMV	*Bird*	*Number*
PPMV-1	pigeons	103
	collared doves	16
	chickens	1
	pet/other birds	9
virulent APMV-1	chickens	3
	psittacines	1
low virulence APMV-1	chickens	8
	ducks	4
	turkeys	9
	pheasants	2
	wild waterfowl	10
	psittacines	3
	pet/other birds	1
	mule ducks	1
	pigeons	3
	goose	1
	guinea fowl	1
APMV-2	caged birds	10
	pheasants	1
	chickens	3
APMV-3	caged birds	20
APMV-4	wild waterfowl	2
	duck	1
APMV-9	wild waterfowl	

SEROLOGY FOR APMV-1

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

ESTONIA

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
Broilers	18	2978	1	10

DENMARK

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
Fowl	1223	36099	181	1550
Ducks and geese	300	3664	9	53
Game birds	52	1279	1	8
Turkeys	93	1180	2	19
Pigeons	30	197	1	3
Ostriches	8	136	2	2
Other birds	16	53	2	7

FINLAND

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
layers broilers turkeys	97	5185	1 [broilers]	29

NORWAY

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
Fowl	118	6384	0	0
Turkeys	11	420	0	0
Domestic geese	5	221	0	0
Domestic ducks	2	120	0	0
Pheasants	1	1	0	0
Mallards	1	59	0	0
Other species	1	1	0	0

SWITZERLAND

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
laying hen		106	0	0
broiler		5	0	0
pet bird	23	23		0
pigeon*	4	16	1	7
feral bird	1	10		
pheasant	1	10		

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	8	790		
Broiler breeders	93	5797		
Imported layer breeders in isolation	7	696		
Layer breeders	36	1812		
broilers	2	110		
Imported turkey breeders in isolation	7	700		
Turkey breeders	7	435		
Backyard poultry	1	44	1	15
Ostriches	6	38		
Wild birds		42		1

The widespread serological surveillance in Denmark was the result of the outbreaks, which are reported elsewhere in these Proceedings. The occasional detection of positive flocks in other countries, probably represents introduction of viruses of low virulence from infected feral birds. Virus isolation attempts on these flocks were usually negative. In Switzerland pigeons may be vaccinated, which probably accounts for the positive sera.

CONCLUSION

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. However, the continued presence of ND in the racing and feral pigeon/dove populations in Europe [an epizootic that now spans 21 years] remains a serious cause for concern and threat to poultry.

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 1999 - 2003:

COUNTRY	1999	2000	2001	2002	2003
Austria	0	0	0	0	0
Belgium	0	0	0	0	**8**
Denmark	0	0	0	0	0
Finland	0	0	0	0	0
France	0	0	0	0	0
Germany	0	0	0	0	**1**
Greece	0	0	0	0	0
Ireland	0	0	0	0	0
Italy	**58**	**351**	0	0	0
Luxembourg	0	0	0	0	0
Netherlands	0	0	0	0	**241**
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	**58**	**351**	**0**	**0**	**250**

1.2. Newcastle disease

Table 2: outbreaks reported by Member States by the ADNS (animal disease notification system 1999 - 2003:

COUNTRY	1999	2000	2001	2002	2003
Austria	4	0	0	0	0
Belgium	0	0	0	0	0
Denmark	0	0	0	135	0
Finland	0	0	0	0	0
France	1	0	0	0	0
Germany	0	0	0	0	0
Greece	0	0	0	0	0
Ireland	0	0	0	0	0
Italy	1	256	1	0	1
Luxembourg	0	0	0	0	0
Netherlands	1	0	0	0	0
Portugal	0	0	0	0	0
Spain	0	0	0	0	0
Sweden	0	0	1	0	0
United Kingdom	0	0	0	0	0
TOTAL	**7**	**257**	**2**	**135**	**1**

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

1.3.1. AVIAN INFLUENZA

The chronology of events and the protection measures taken on Community level during the epidemic of avian influenza in the Netherlands, Belgium and Germany in 2003 can be visited on the following website:

http://europa.eu.int/comm/food/animal/diseases/controlmeasures/avian/chronology_2003_epidemic.pdf

1.3.1.1. The Netherlands

The first strong suspicion of an outbreak of highly pathogenic avian influenza was reported to the Dutch authorities on 28 February 2003 in six holdings, mainly in laying hens in free range in an area called “Gelderse Vallei” situated in the central province of Gelderland. The Dutch veterinary services informed the Commission that evening and implemented a total standstill

for all movements of poultry until Monday 3 March. Highly pathogenic avian influenza of subtype H7N7 was subsequently confirmed.
Commission Decision 2003/153/EC on protection measures in relation to a strong suspicion of avian influenza was adopted on March 3 on the Commission's initiative. The Standing Committee on the Food Chain and Animal Health which was called in for a meeting on 5 March confirmed these safeguard measures. The measures foreseen in Council Directive 92/40/EEC on Community measures for the control of avian influenza such as stamping out of poultry flocks infected, suspected of being infected or contaminated, the establishment of protection and surveillance zones around confirmed outbreaks and restrictions for movements of live poultry and poultry products were implemented by the Dutch authorities. These measures were complemented by an export ban for live poultry and hatching eggs from the whole territory of the Netherlands and a ban of transport of live poultry and hatching eggs within the territory of the Netherlands except for the transport of poultry for immediate slaughter and day-old chicks to holdings under official supervision.
All these measures were endorsed by Commission decisions which have always obtained unanimous support by the Member States. During the meetings of the Standing Committee, which usually meets once a month, the Dutch authorities reported to the Commission and the Member States on the evolution of the disease situation and the control measures taken. Additional eight meetings were organised with Member and the 10 Acceding States to review the situation.
Aiming to create poultry free zones bordering the infected areas to let the infection "die out", all poultry farms and other poultry kept at risk in defined areas (so-called "buffer zones") were depopulated from 23 March (endorsed by Commission Decision 2003/214/EC). Despite these aggressive control and eradication measures to contain the disease the infection was also detected on 3 April in the province of Limburg in the south east of the Netherlands close to the border of Germany.
Further measures taken by the Dutch authorities were a nation wide serological screening of poultry flocks to assess the spread of the disease and, if low pathogenic avian influenza had been circulating in the area, the tightening of biosecurity measures in all sectors of the poultry production chain and the forming of geographical "compartments" aiming in particular to avoid risky contacts of poultry, means of transport, equipment and people entering or leaving farms, egg packing stations, hatcheries, slaughter houses, feed mills, litter processing and rendering plants. For this purpose all poultry farmers had to keep registers for their professional visits to their farms as well as their professional visits to other poultry holdings (endorsed by Commission Decision 2003/258/EC).
The last outbreak in a commercial flock was detected on 29 April and in a hobby flock on May 3. A total of 241 outbreaks were confirmed in the Netherlands (226 on commercial farms and 15 on backyard flocks).Approximately 30 million heads of poultry were killed on infected farms or were killed pre-emptively (1.400 holdings in total).
Following final cleaning and disinfection sentinel birds had to be placed in holdings that were previously found infected and had to be tested after 21 days with negative results for avian influenza before restrictions could be lifted for the surveillance zones (Commission Decision 2003/428/EC). The last restrictions for the Netherlands were lifted on 22 August and the disease considered as eradicated.

1.3.1.2. Belgium

A first suspicion was raised on March 11 2003 in the province of Antwerp (Commission Decision 2003/173/EC). The suspicion was ruled out and the protection measures repealed on 18 March 2003.
On 16 April another strong suspicion was reported to the Commission in the province of Limburg, which was subsequently confirmed as highly pathogenic avian influenza of the Dutch subtype H7N7 (Commission Decision 2003/275/EC). A total of 8 outbreaks occurred in the provinces of Antwerp and Belgium. There was no reported spread to non-commercial poultry flocks. The last outbreak was detected on 28 April. The Belgian authorities implemented the control measures according to Council Directive 92/40/EEC and additional measures similar to the measures taken by the Dutch authorities. Approximately 3,2 million heads of poultry were killed on infected farms and such holdings which were emptied as a preventive measure. Following the procedures as provided for in Decision 2003/428/EC with negative testing results the last restrictions on Belgian poultry farms were lifted on 15 July. However, heightened vigilance and biosecurity measures continued to apply.

1.3.1.3. Germany

Only one outbreak of avian influenza H7N7 subtype was confirmed on the German territory in Schwamtal, in the county of Viersen, in the Land of North Rhine Westphalia close to the infected Dutch province of Limburg on 13 May. Commission Decision 2003/333/EC was adopted and introduced protection measures for the whole Land of North-Rhine Westphalia. Indirect contacts via persons were considered the most likely way of introduction of disease into this holding. Approximately 450.000 heads of poultry were killed and the disease was considered eradicated by 25 June, when all restrictions were lifted.

1.3.1.4. Additional measures taken in relation to avian influenza on pig holdings

Following an expert group meeting held at the Commission obligatory laboratory investigations and temporary movement restrictions were introduced by Commission Decision 2003/290/EC in “mixed holdings”, where poultry and pigs were kept on the same farm in order to assess their possible role for the transmission of the disease to other animals or humans. In the Netherlands pigs on five farms, where poultry had been found infected showed clear serological evidence of infection with the avian influenza strain subtype H7N7. Further PCR and virus isolation tests were negative suggesting that the pig’s role for spreading the infection might be transitory and can be considered as rather insignificant in particular once the infected poultry has been removed from the farm.

1.3.1.5. Authorisation of vaccination against avian influenza of susceptible birds in zoos and approved bodies, institutes or centres

By Commission Decisions 2003/291/EC and 2003/359/EC competent authorities were entitled to authorise vaccination against avian influenza in zoos and approved bodies, institutes or centres for rare breeds of poultry and birds. This vaccination was practised under official control with obligations for the keeper to identify the birds and to keep a register. Movement restrictions for vaccinated birds had to be put in place.

1.3.1.6. Public health aspects

In the Netherlands, 82 human cases of conjunctivitis and/or ILI (influenza like illness) due to avian influenza virus occurred in workers directly exposed to infected birds. Some secondary cases also occurred in relatives who lived in contact with the affected personnel. On 17 April a veterinarian died of pneumonia after visiting an affected farm. Following the *post-mortem* examination, the Dutch authorities arrived to the conclusion that his death was most likely linked to the circulating avian influenza virus. Commission Decision 2003/290/EC required that the competent authorities shall ensure that appropriate precautionary measures are adopted as regards the prevention of influenza infections in poultry workers and other persons at risk, which may include the use of protective clothing, gloves and goggles, vaccination against human influenza with a view to lower the risk of possible re-assortment of genetic material of human and avian influenza viruses and prophylactic antiviral treatment.

1.3.1.7. Financial aspects

According to Council Decision 90/424/EEC on expenditure in the veterinary field the Community reimburses Member States up to a maximum of 50% of the costs they have incurred for compensating owners for the killing of the poultry and the destruction of their products, cleaning and disinfection of holdings and the destruction of contaminated feed and equipment.
In the Netherlands these direct losses are approximately 160 million Euro, in Belgium 10 million and in Germany 1 million Euro, which shall be eligible for co-financing. The financial contribution by the Community is only granted after examination of the submitted dossiers provided that their control is satisfactory and that disease control measures have been implemented in full compliance with Community legislation.

1.3.2. LEGISLATION IN RELATION TO LOW PATHOGENICITY AVIAN INFLUENZA (LPAI) IN ITALY

Following the re-emergence of LPAI infections of subtype H7N1 a vaccination programme was approved by the Commission by Decision 2000/721/EC of 7 November 2000 to supplement the measures to control avian influenza including specific restriction measures for trade. The vaccination strategy followed the DIVA approach (allowing a differentiation between infected and vaccinated animals by using a vaccine strain different from the circulating field strain, sentinel birds and appropriate discriminatory testing). At the onset of this campaign restrictions were put in place on all live poultry and poultry products originating from the vaccination area. The vaccination programme that was carried out under the official control of the competent veterinary authority and with accompanying intensive surveillance on vaccinated and non-vaccinated poultry flocks proved to be successful.

In October 2002, following the introduction of a new avian influenza subtype H7N3, a new vaccination programme was adopted by Commission Decision 2002/975/EC of 12 December 2002 for a larger area covering parts of the regions of Veneto and Lombardia. The results of the vaccination programme reported at several meetings of the Standing Committees on the Food Chain and Animal Health were generally favourable in view of the control of the disease within the vaccination zone. However, the infection had spread to some areas adjacent to the established vaccination zone which made it necessary to extend the vaccination area by June 2003. By respecting intensive surveillance and testing procedures on vaccinated poultry

flocks and the experiences gained in this respect it was possible to ease the trade restrictions and to authorise intra-Community trade in fresh meat from vaccinated turkeys and chickens and of table eggs from vaccinated hens. Since the end of September 2003 no further circulation of LPAI has been detected in the area.

1.3.3. NEWCASTLE DISEASE (ND)

1.3.3.1. Denmark

Denmark (like Sweden and Finland) does not practise prophylactic vaccination against ND in its poultry flocks. The non-vaccinating status of Denmark is officially recognised by Commission Decision 91/552/EC. To maintain this status annual serological testing of breeding flocks according to Decision 94/327EC must be performed with negative results. This status allows these Member States to require additional testing and certification when trading in live poultry and hatching eggs from a country or Member State that carries out vaccination against ND.

On 15 July 2002 a layer breeding farm in Nordjylland was placed under official surveillance due to a first clinical suspicion of Newcastle disease. By 26 July laboratory confirmation of Newcastle disease by ICPI testing was obtained. Measures according to Council Directive 92/66/EEC on Community measures to control ND were introduced and the dispatch of live poultry and hatching eggs from the whole Danish territory to other Member States and third countries was prohibited.
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 on Community level.
In total 135 outbreaks were detected in 9 commercial holdings and 126 hobby flocks in 8 (out of 14) Danish counties. The "specific trade patterns" between the commercial sector and the hobby flocks made the epidemiological investigations for the Danish authorities rather onerous. As a consequence obligatory registration of small hobby flocks was introduced. A total of approximately 175.000 heads of poultry were killed. The last outbreak was confirmed on 28 August 2002 and the last restrictions on Danish poultry farms were lifted by 1 January 2003.

1.3.3.2. Italy

On 23 July 2003 the Italian authorities notified an outbreak of ND in the region of Palermo, Sicily, to the Commission and Member States. 10 day-old chicks had been purchased by a private household from a local trader and were kept on a terrace in the centre of Palermo city. No other poultry had been kept there. When the chicks started dying 2 weeks later, samples were sent to the ND national laboratory in Legnaro and ND was confirmed. On 1 July all chickens had died and final cleaning and disinfection was concluded on 17 July. Protection and surveillance zones according to Council Directive 92/66/EEC were established and clinical examinations of poultry holdings in these zones revealed no further suspicion of disease. All restrictions could be lifted 30 days after final disinfection.

1.3.4. Velogenic paramyxovirus-1 infections in wild birds in Denmark

In October 2002, the Danish Veterinary and Food Administration reported to the Commission that PPMV-1 was diagnosed in pheasants on the island of Lolland, south of Zealand. The birds had been released for hunting purposes in July 2002. Since mid September increased mortality had been observed and the isolated virus strains showed an ICPI ranging between 0.65 and 1.1. The birds had originated from 2 hatcheries situated in the northern Jutland, which where both tested serologically and found negative. Hunting was intensified and shot birds safely disposed off.
Council Directive 92/66/EEC introducing Community measures for the control of Newcastle disease does not apply where ND is detected in wild free living birds; however, in that case the Member State concerned shall inform the Commission of any measure it takes.

1.4. LEGISLATION IN RELATION TO SURVEYS IN POULTRY AND WILD BIRDS

In order to assess the occurrence of low pathogenicity avian influenza of subtypes H5 and H7 within the Community and following the recommendation of the opinion delivered in the scientific report on avian influenza from 27 April 2000, Member States were required by Decision 2002/649/EC of 5 August 2002 to submit to the Commission programmes for surveillance for avian influenza in poultry. Investigations in wild birds on a voluntary basis should also be undertaken. Co-financing by the Community up to a maximum of 50% to the costs incurred by Member States for sampling and laboratory testing was decided. A total amount of 600.000 Euro for the 15 Member States was allocated for this purpose. The Community Reference Laboratory together with Member States' experts and the Commission drew up the general guidelines to be followed.
By Commission Decision 2002/673/EC the individual programmes were approved after examination by the Community Reference Laboratory and the Commission. The Community Reference laboratory assisted Member States by supplying antigens for laboratory testing and compiled the results of this first EC-wide screening on LPAI viruses H7and H5 in a report (see relevant part of proceedings).

1.5. INTERNATIONAL TRADE RULES FOR AVIAN INFLUENZA (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 current chapter on highly pathogenic avian influenza needs to be updated with respect to the importance of low pathogenicity avian influenza. A working group met in October 2002 and developed a new chapter on AI. Freedom from AI infection (LPAI and HPAI) shall be demonstrated on basis of an ongoing active surveillance. In case of infection trade shall not be interrupted if freedom from infection can be demonstrated for defined geographical regions or specific compartments of the poultry sector. Compartments are defined by the same management forming an epidemiological entity for the purposes of trade.
The European Commission establishes working groups which comment on the OIE proposed changes to the Code. These are followed by working groups in the Council which endorse these comments are amend them as appropriate. At the OIE's yearly general sessions in May each year (71st General Session in May 2003) changes are discussed, adopted or their

adoption refused. 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

The European Union has up to now been represented at the OIE by its Member States, since 5 December 2003 it has obtained an official observer status.

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

2.1. Avian influenza in Chile

On 31 May 2002 Chile began sending regular information to the OIE and the EU concerning a suspicion of avian influenza in a broiler breeding farm in Valparaiso, province of San Antonio, fifth Region. Increased mortality had been noted, the holding was placed under official surveillance and movement restrictions were put in place in a 10 km radius. On 25 May low pathogenicity avian influenza of H7N3 subtype was diagnosed. About 400.000 birds were killed as a precautionary measure and hatching eggs destroyed.

In 2000 a nation wide survey had been carried on about 70.000 samples. No positive serology for AI had ever been detected.

On 1 July 2002 highly pathogenic avian influenza of subtype H7N3 was confirmed in the suspected holding and a second farm of turkey breeders exhibiting clinical signs located at 4 km distance. It appeared that mutation to highly pathogenic AI had occurred in one poultry establishment.

On 21 June 2002 Chile had suspended certification for exports of live poultry, hatching eggs and poultry meat to the EU. Furthermore the EU and Chile were in the process of signing a Veterinary Agreement based on mutual confidence in the veterinary services of each trading partner. Therefore safeguard measures on EC level were not deemed necessary at that stage. However, one Member State introduced unilateral protection measures, the situation was reviewed at the Standing Committee on the Food Chain and Animal Health and Commission Decision 2002/607/EC of 23 July was adopted prohibiting the importation of live poultry, hatching eggs, live ratites, fresh meat of poultry, ratites, farmed and wild feathered game, poultry meat products and meat preparations consisting or containing meat of the abovementioned species from the whole territory of Chile. Derogation was granted for heat treated meat (up to 70°Celsius throughout the product) and for poultry meat that had been obtained from poultry slaughtered before June 21. The Chilean veterinary authorities provided detailed documentation on tracing for poultry meat.
After the first two outbreaks no further outbreak was detected. Following stamping out measures on infected farms, cleaning and disinfection and the performance of two nation wide screenings for AI, (the second in August 2002), the restriction measures for trade were reduced by 18 October 2002 by Commission Decision 2002/796/EC amending Decision 2002/607/EC introducing regionalisation of Chile with the exception of a defined area within region V of Chile.

The veterinary authorities of Chile requested support to the OIE for their disease control measures. Between 17-25 July 2002 Dr. Ilaria Capua and Dr. Stefano Marangon, two experts from the OIE reference laboratory in Legnaro, Italy, which is also the national laboratory for AI/ND in Italy, evaluated the situation in Chile and gave recommendations based on their own vast experience with AI epidemics.

It must be noted that the Chilean authorities have displayed great transparency in the disease control measures applied. This proactive communication policy has enabled trading partners to get a clear picture of the disease situation and its evolution and therefore allowed a swift reduction of trade restrictions.

2.2. Newcastle disease in Australia

Since 1998 Australia has experienced re-occurring ND outbreaks in New South Wales and Victoria. The disease was caused by a paramyxovirus-1 strain of low virulence that had been endemic since the mid sixties in Australian poultry flocks but which had acquired virulence. However, in many cases clinical signs were not significant and mortality rates remained rather low. The last outbreak was recorded in New South Wales in November 2002.
EC-safeguard measures had been in place since the end of the nineties and amended several times. The original non-vaccination policy for ND practised in Australia was changed and vaccination introduced. When safeguard measures elapsed Commission Decision 2003/810/EC of 17 November 2003 was adopted amending testing and certification procedures in relation to imports of fresh poultry meat, farmed ratite meat, live ratites and hatching eggs from third countries with respect to Australia. The main imports from Australia concern ratite meat. The farms of which the slaughter birds are obtained must undergo regular testing for ND. To assess the situation on the spot an inspection of the Food and Veterinary Office in scheduled for January 2004.

2.3. Newcastle disease in the USA

On 9 October 2002 ND was reported in a non-commercial flock in the urban area of Los Angeles, in the State of California.

Subsequently the disease spread to more than 1000 holdings in southern California. Also 22 commercial holdings became infected.
To a limited extent the disease also spread to the neighbouring States of Nevada and Arizona, where it was quickly brought under control. Also the State of Texas encountered an outbreak of ND in a hobby flock near El Paso, close to the Mexican border. Areas of Texas and the State of New Mexico were placed under quarantine.
Certification from the whole territory of the USA had been suspended since confirmation of disease, as animal health certificates accompanying consignments of live poultry, hatching eggs and poultry meat require that the official veterinarian certifies that the USA is officially free from AI and ND. The United States remained blocked for exportation of live poultry and poultry products until Council Decision 2003/67/EC of 28 January came into force and limited the trade restrictions to the States of California, Nevada and Arizona. Following the

outbreak in Texas on 11 April the decision had to be amended to include the infected area of Texas and a neighbouring area of New Mexico.
On May 31 2003 the last outbreak was recorded. From 19-29 May an inspection team of the Food and Veterinary Office visited the United States to assess the implementation of disease control and regionalisation measures.

Newcastle Disease (ND) Outbreak in the United States: 2002-2003

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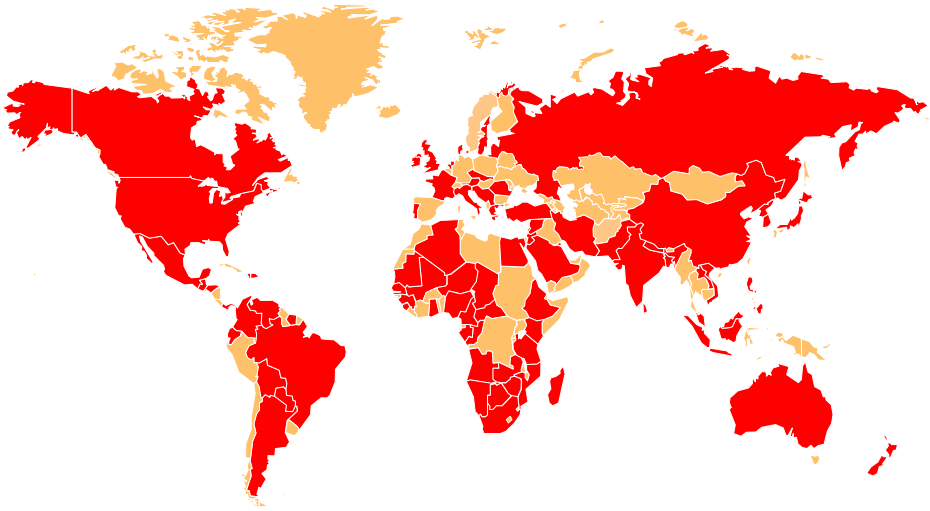
Objectives

- Background
- Brief review of the outbreak
  - ✓Backyard game fowl
  - ✓Commercial poultry
- Management of the outbreak - ICS
- Diagnostics/virus characterization
- Long term goals

Reports of ND to OIE, 1997-2000



● Countries positive (106)

Newcastle Disease (ND): *OIE Definition*

- NDV is a member of the *Avulavirus* genus, *Paramyxoviridae* family
- Caused by avian paramyxovirus type 1 (APMV-1)
  - ✓ICPI: >0.7
  - ✓Multiple basic amino acids at fusion cleavage site and F at position 117 (N terminus of F1)
- In the USA, virulent forms = vND, exotic Newcastle disease (END)

Recent Isolations of vNDV in North and Central America

- 1992 – United States – turkeys (ND)
- 1998 – United States – BY game fowl (CA)
- 2000 – Mexico – chickens
- 2000 – Honduras – chickens
- 2001 – Mexico – fighting cocks
- 2002-03 – United States – chickens, BY game fowl, wild birds, pet birds

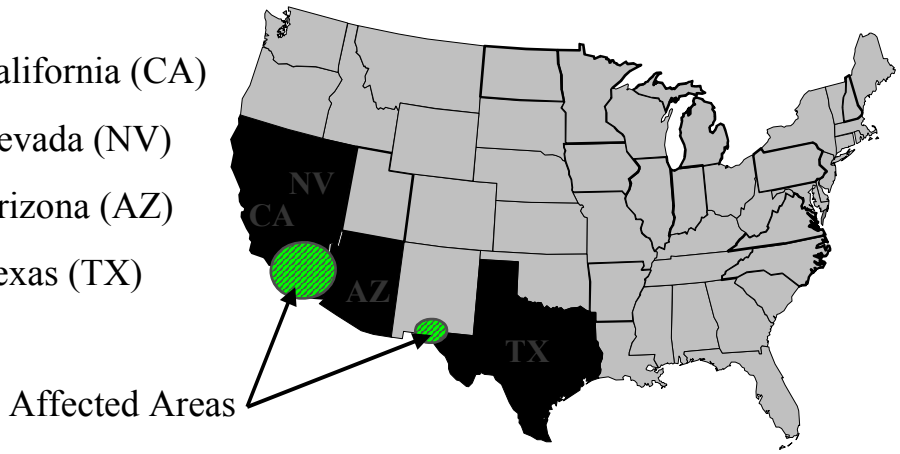
2002/2003 vND Outbreak: *Affected States, Regions*

California (CA)

Nevada (NV)

Arizona (AZ)

Texas (TX)



2002/2003 vND Outbreak: *The Agent*

- Avian paramyxovirus type-1
- ICPI = 1.75
- Fusion protein cleavage site sequence:
  - ✓RRQKR/FVG, RRQRR/FVG (2 isolates)
- CA, NV, AZ isolates indistinguishable
- Texas outbreak likely a separate introduction
- U.S. isolates similar to recent vND isolates from Mexico

Early Events – May, 2002

- vND diagnosed in ring-necked parakeets with connections to a Southern California swap meet
- Tracebacks did not detect further infections
- Virus indistinguishable from outbreak isolates

Early Events – September, 2002

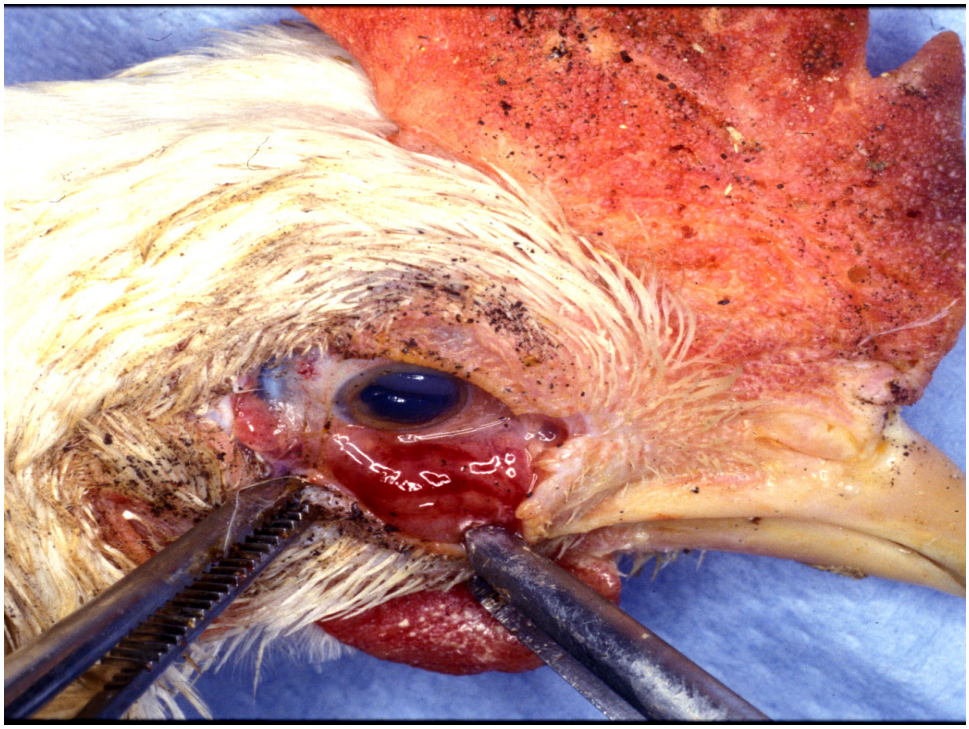
Index Case:

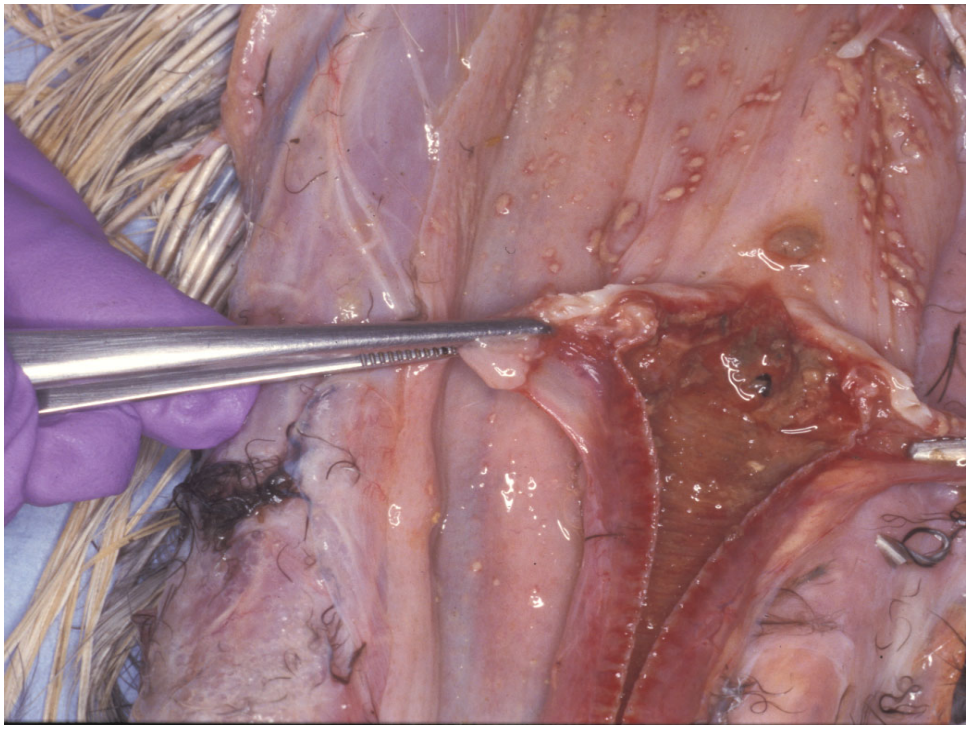
- Submitted: 9/25/02
  - ✓ Urban, Los Angeles
- Submitter: private practitioner
- Complaint: mucoid discharge from n
  breathing, swollen sinuses, 8-10 died out of 200
- Suspected diseases: Coryza, Mycoplasma
- vND confirmed at the NVSL: October 1, 2002

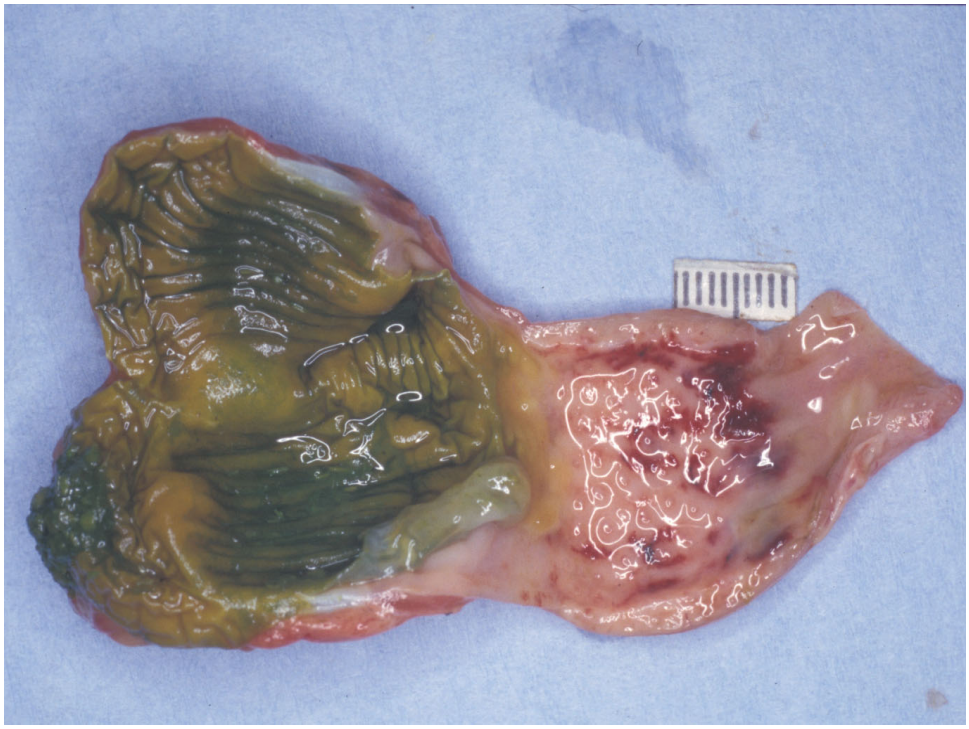
General Features of the Outbreak

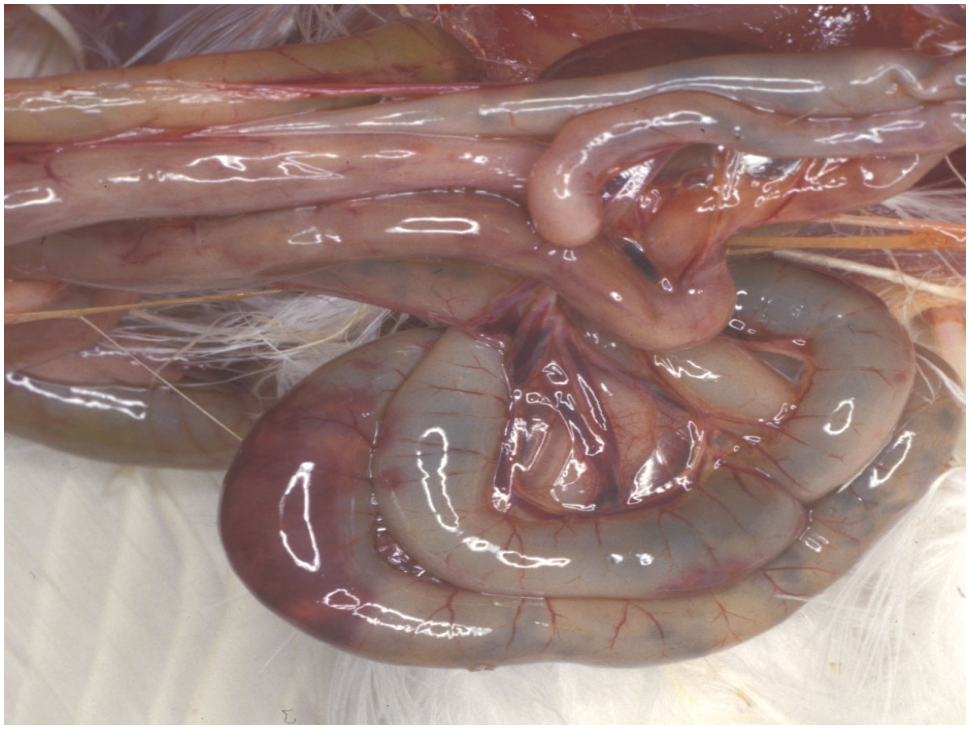
- **Backyard game fowl:** high morbidity and mortality

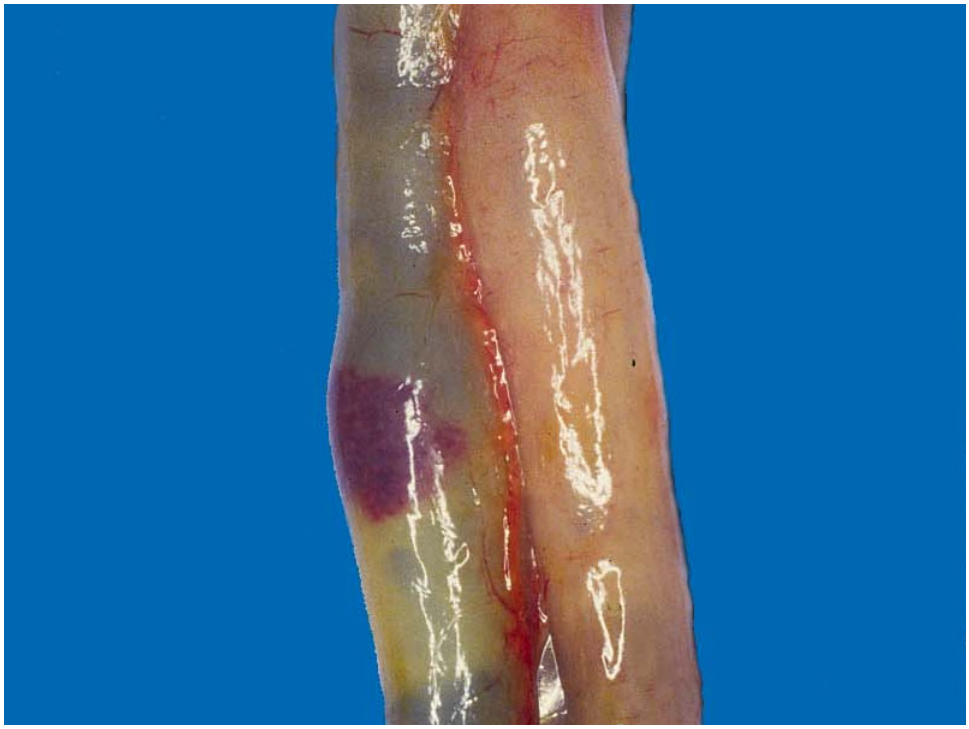
- **Commercial layers:** low morbidity and mortality
- Very few cases involving pet birds, ratites, ducks, pigeons (wild and domestic) etc.

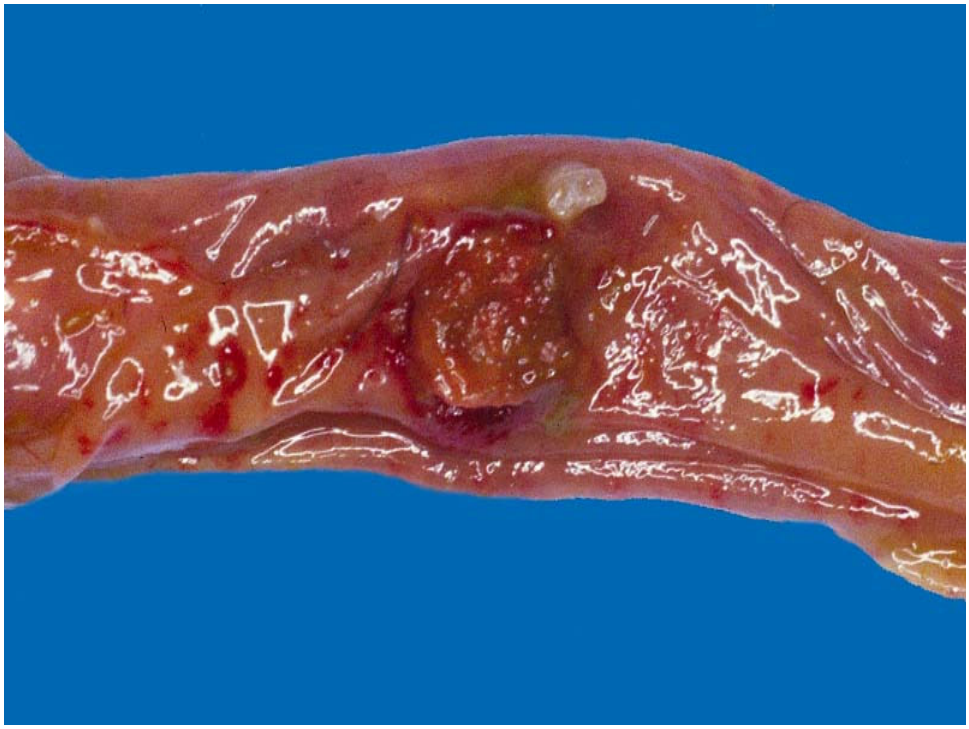


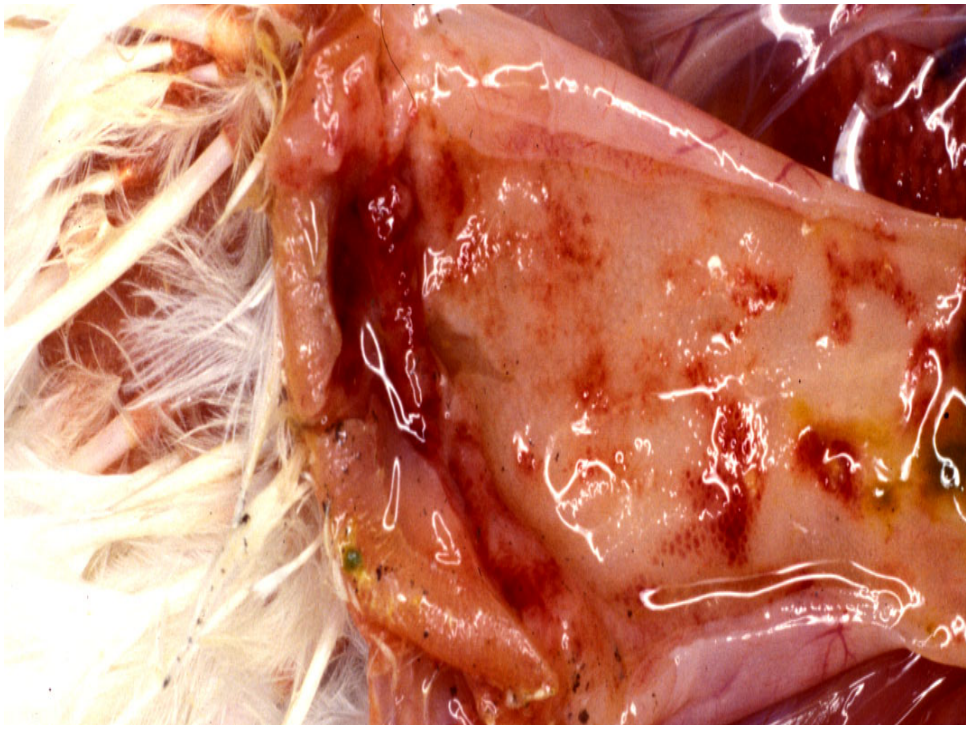










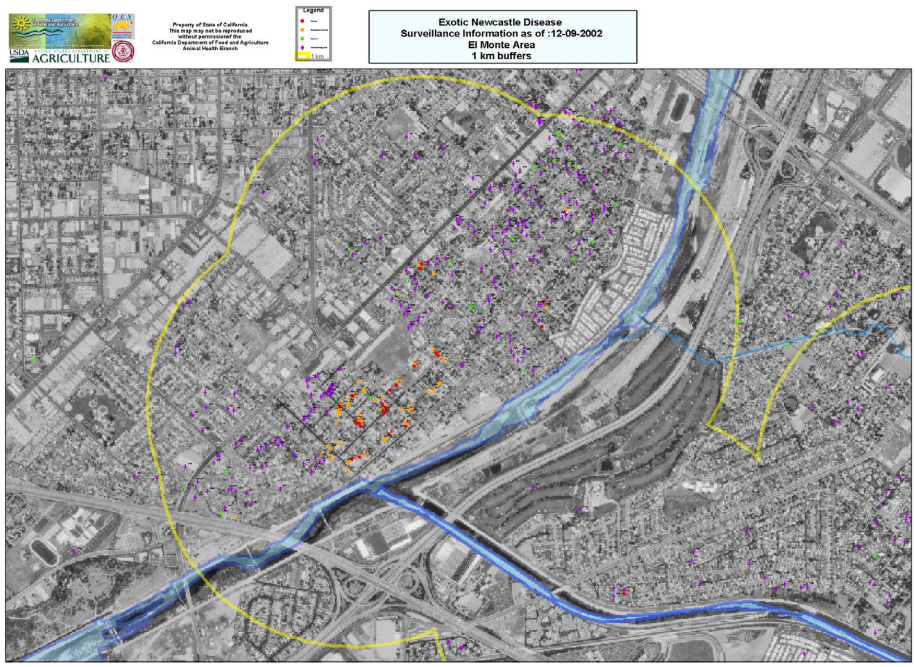


2002-2003 vND Outbreak: *New Challenges*

- Cultural barriers
- Defining population at risk
- Disease detection (Dx)
- Ongoing animal movement
- General lack of biosecurity, good management practices

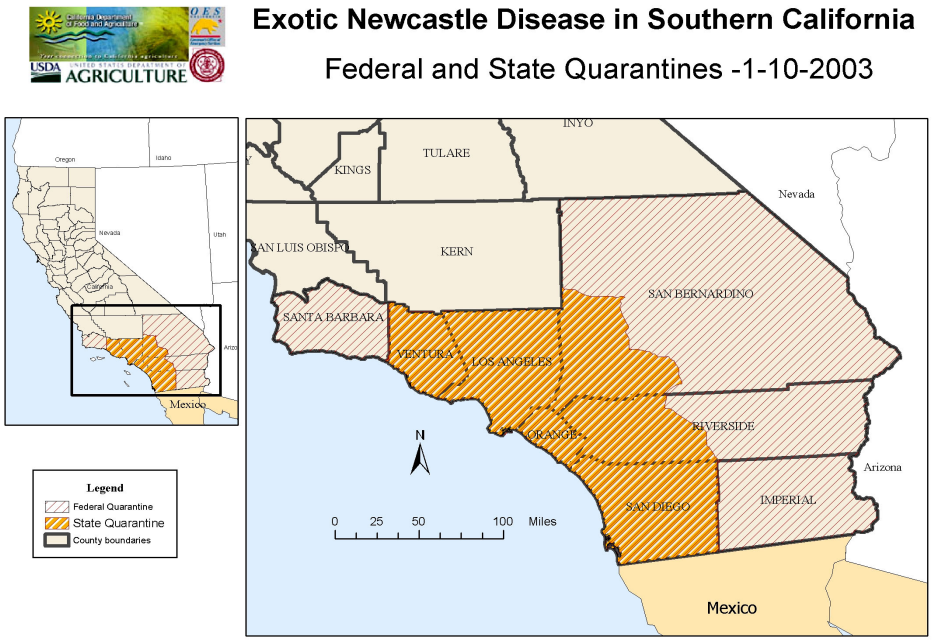
Cultural Traditions


Urban Interface


Property of State of California
This map may not be reproduced without permission of the California Department of Food and Agriculture Animal Health Branch
Legend
Exotic Newcastle Disease
Surveillance Information as of :12-09-2002
El Monte Area
1 km buffers

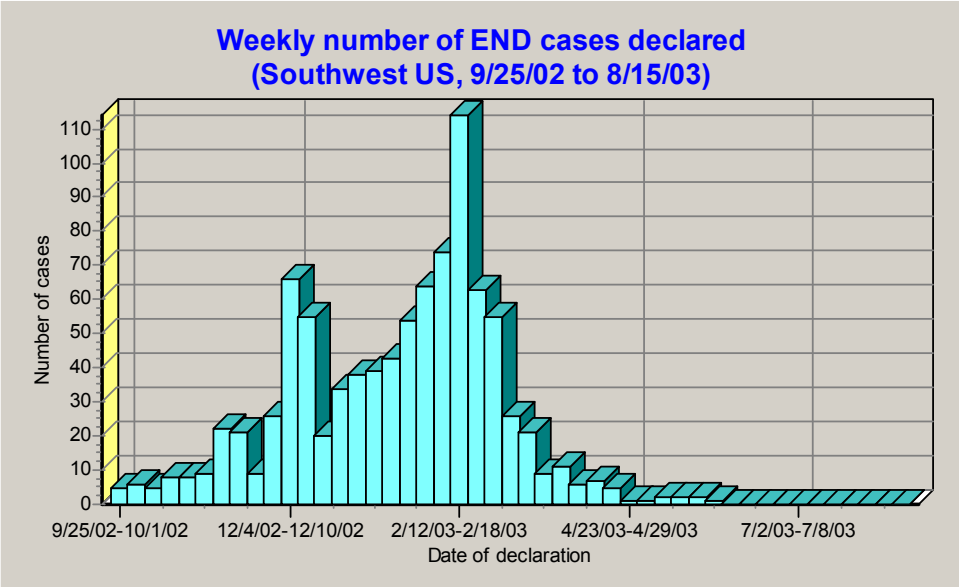
2002/2003 END Outbreak:
(Backyard - CA, NV, AZ, TX)

- 2,671 premises positive or DC
- 149,247 birds depopulated
  - ✓>10 species infected (chicken, pigeon, duck, goose, quail, guinea fowl, pheasant, emu, owl, unspecified)
- 19,056 premises quarantined
- 200,000 premises surveyed



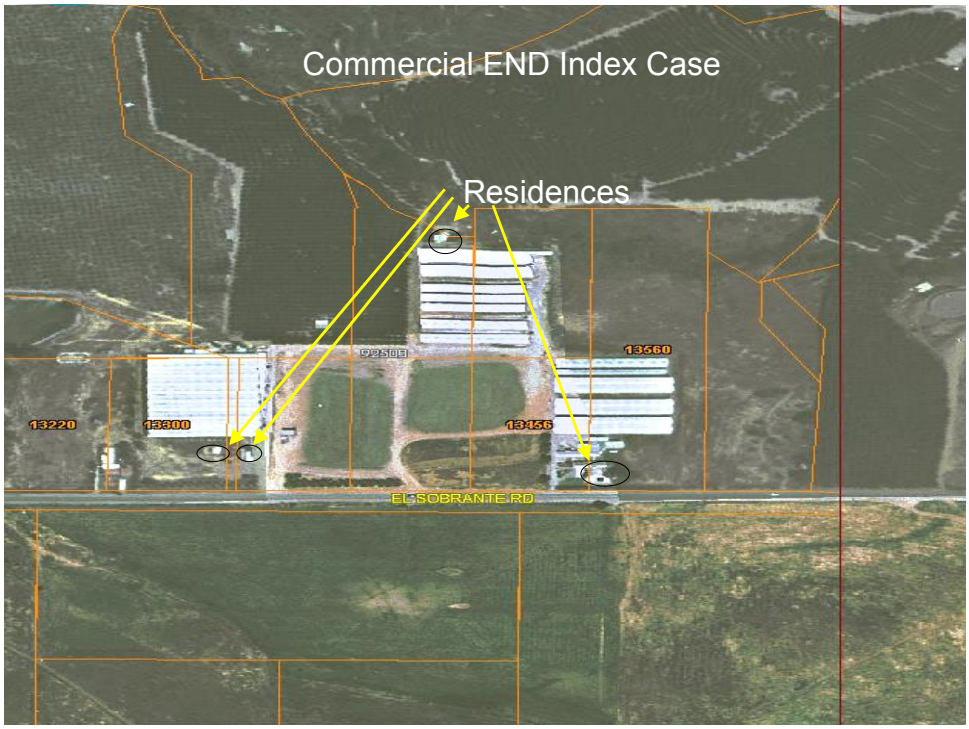
Additional Setbacks

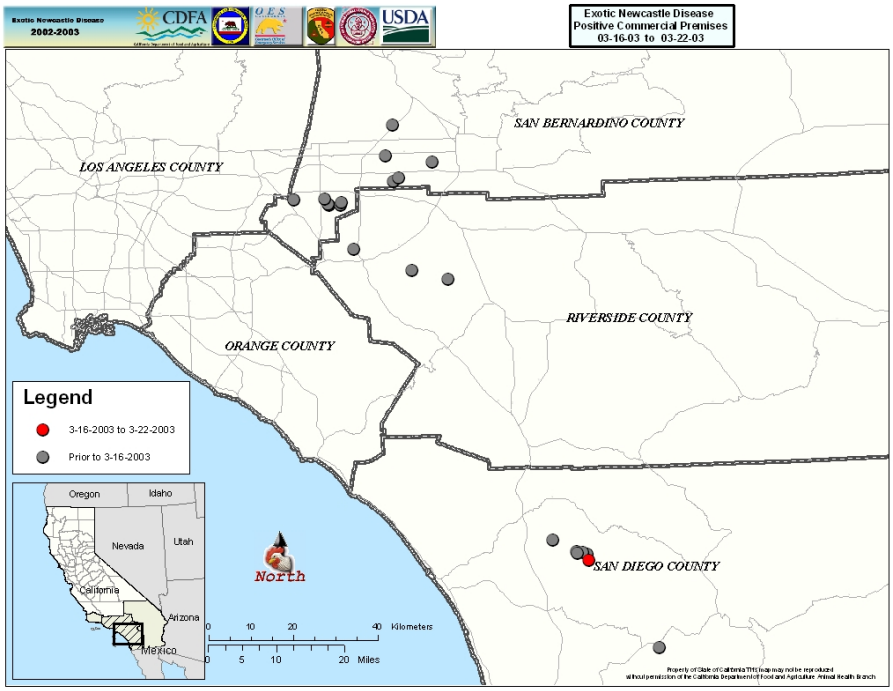
- January 16, 2003 – Nevada (2 counties)
  - ✓138 (10 IP) premises depop (2,746 birds)
  - ✓Jan 29 – last positive case
- February 4, 2003 – Arizona (3 counties)
  - ✓4 (1 IP) premises depop (269 birds)
  - ✓Feb 7 – last positive case
- April 9, 2003 – Texas (2 counties, 3 in NM)
  - ✓40 (1 IP) premises (2,002 birds)



END in California:
Commercial Poultry

- 12/19/02 – first positive submission
- Table egg layers
- Challenges:
  - ✓Subtle effects on production and clinical signs
    - ➢Drop in egg production – 5-10%
    - ➢Increased mortality – 0.7-5%
  - ✓Concurrent disease in Southern California (H6N2)
  - ✓Close contact with BY birds





2002/2003 END Outbreak: *(Commercial - CA)*

- 21 premises positive + 1 DC
- >3.2 million birds depopulated
- 3/26/03 – last positive case
- 103 flocks in southern CA under active surveillance

Commercial Outbreak Dynamics

Risk Factors

- Employees
- Proximity to infected backyard poultry
- Egg flats, racks
- Movement of manure
- Wildlife

Firewalls

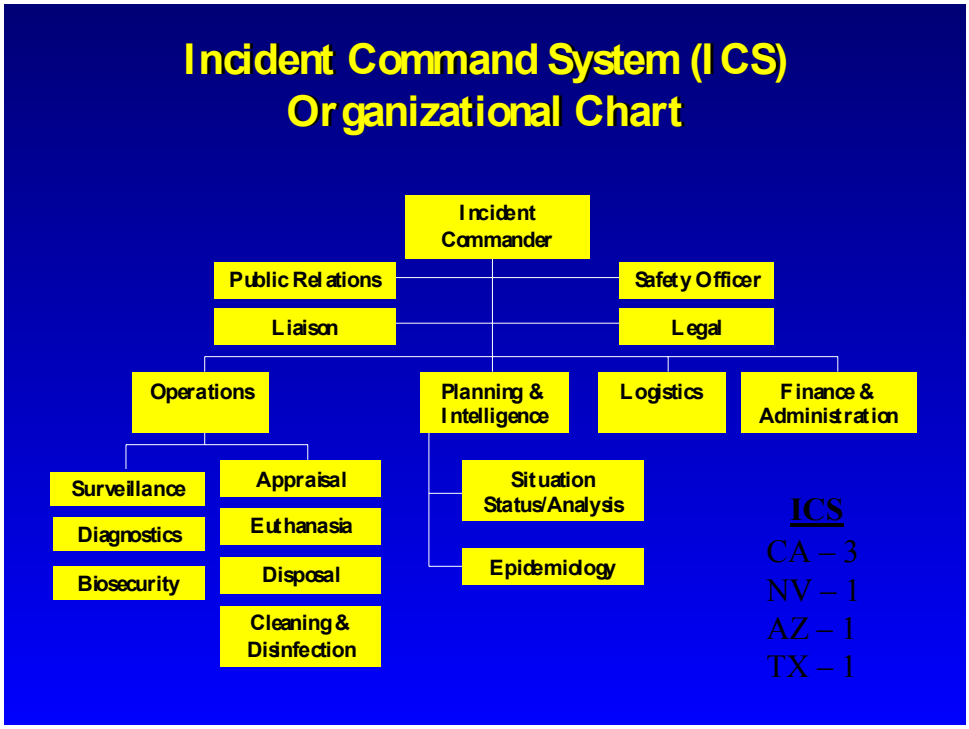
- Protective clothing, employee training
- Egg flats, cleaning and disinfection
- Backyard monitoring
- Controlled access

2002/2003 END Outbreak: *Key Epidemiologic Factors*

- Pet birds – smuggled
- Backyard game fowl – smuggled
- Co-mingling of pet and backyard birds
- Large susceptible population of backyard birds
- Highly mobile population – owners/birds
  - ✓ Within state and out-of-state
  - ✓ Feral and free-ranging birds
- People/contaminated meat product, equipment?
- Employee interaction – backyard/commercial flocks
- Insufficient biosecurity in commercial industry

2002/2003 ND Outbreak: *Costs and Indemnities Paid*

- Direct expenditures – $192 million
- Indemnities (BY game fowl)
  - ✓Range: $5 - $300
  - ✓Average: $140/bird




Incident Command Post – Garden Grove, CA

Personnel

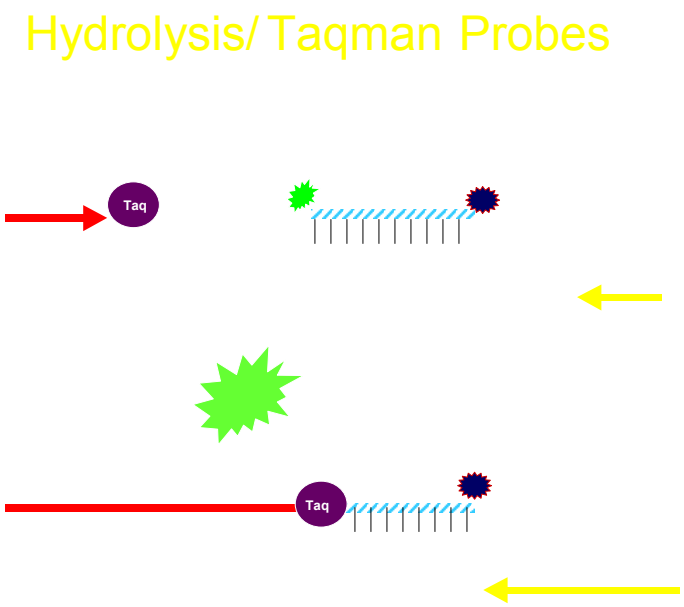
- Approximately 8,000 people rotated through the ICS
  - ✓ 21 day rotations

Laboratory Diagnosis

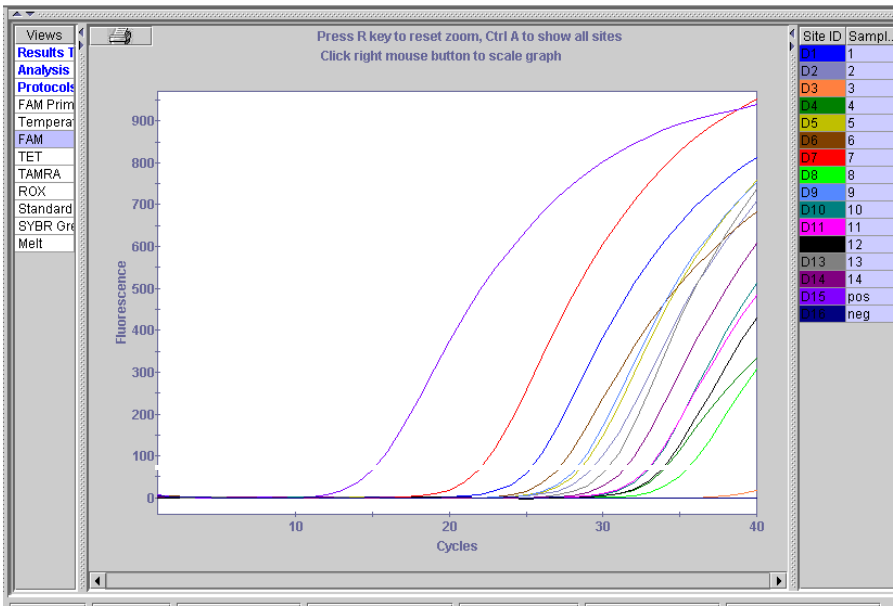
- Virus isolation (2 – 12 days)
  - ✓CDFA labs – San Bernardino, Fresno
  - ✓NVSL
- Real Time RT-PCR (RRT-PCR)
  - ✓Cooperative effort (SEPRL, UC-Davis, NVSL)
  - ✓Extensive validation – 1,500 specimens (300 positive, 1,200 negative)
  - ✓Same day test results (2.5 hr)

Laboratory Diagnosis

- Real Time RT-PCR (RRT-PCR)
  - ✓Matrix primers/probe – all APMV-1 strains
    - ➢DxSen = 96.7%, DxSp = 97.3%
  - ✓Cal/Mex primers/probe – vNDV
    - ➢DxSen = 92.9%, DxSp = 99.1%
- RRT-PCR replaced virus isolation – April 22
- Over 100,000 RRT-PCR tests performed

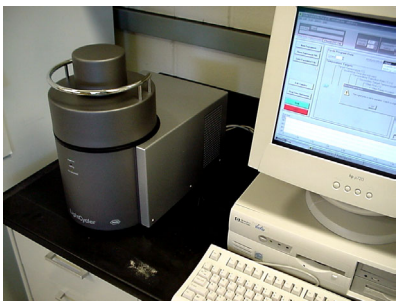


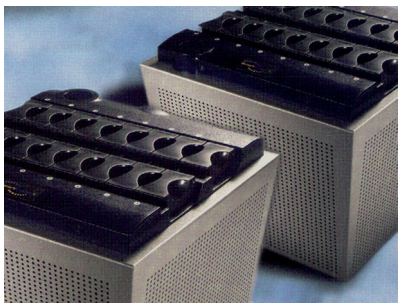
Erica Spackman (SEPRL, Athens, GA)



Molecular Diagnostics

- **Real Time RT-PCR (RRT-PCR)**
  - ✓Smart Cycler System – Cepheid, Sunnyvale, CA
  - ✓LightCycler System (Roche Molecular biochemicals, Indianapolis, IN)





Sample Collection: *Tissue versus Swabs*

- Case study of 162 submissions
- Tissue – no advantage over swabs
  - ✓Tracheal swabs – 100% sensitivity
  - ✓Oropharyngeal swabs – 99.8% sensitivity
  - ✓Cloacal swabs – 95.6% sensitivity
- Tracheal swabs – specimen of choice
  - ✓Easy to collect
  - ✓Easy to process for VI & RRT-PCR

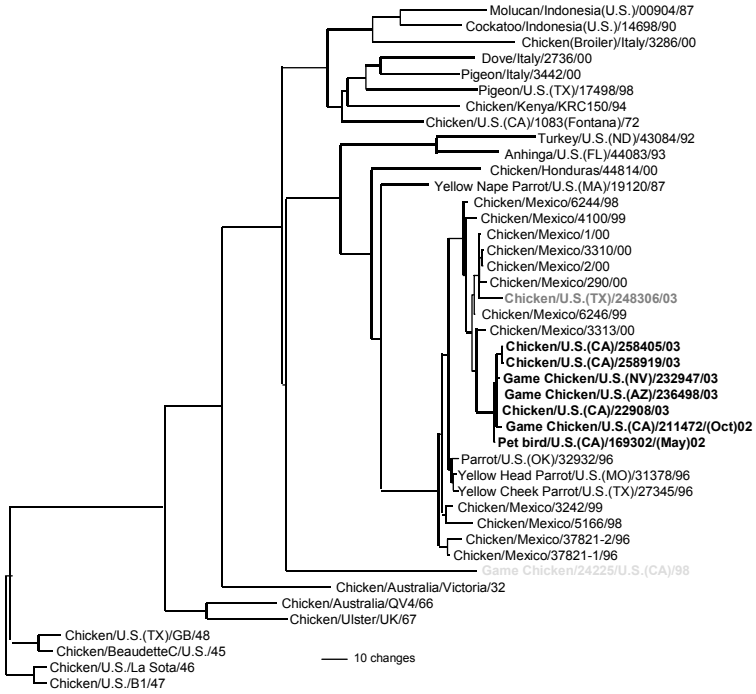
Virus Characteristics

- Isolates from CA, NV, and AZ are indistinguishable
  - ✓ICPI 1.73-1.75 (n=28)
  - ✓A.A. sequence = **RRQKR/FVG** (n=274)
  - ✓A.A. sequence = **RRQRR/FVG (**n=2); A ⟶ G
- TX vNDV likely a separate introduction
  - ✓ICPI = 1.83
  - ✓A.A. sequence = **RRQKR/FVG** (97% similar)
  - ✓Mab binding pattern different than CA/NV/AZ

Virus Characterization: *Monoclonal Antibody Testing*

Monoclonal Antibodies (HI)

Virus	B-79	AVS-1	15C-4	10D11C	161-617
CA/NV/AZ	+	-	+	-	-
TX	+	-	-	-	+



Post Outbreak Surveillance

- Last Positive Flock – May 31, 2003
- Infected Zones
  - ✓95% confidence – 0.5% infection
- Non Infected Zones
  - ✓95% confidence – 1% infection
- Actual testing = 200% of target CI

Long Term Goals

- National END surveillance plan
- National Avian Health Mitigation Group to reduce risk of introducing non endemic diseases (30 new positions in CA)
  - ✓Outreach/education
  - ✓Surveillance
  - ✓Biosecurity
  - ✓Research


Biosecurity Training

Acknowledgements

- Dr. David Castellan (CDFA)
- Dr. Hailu Kinde (CDFA)
- Jan Pedersen (NVSL)
- Dr. Jack Shere (APHIS)
- Dr. Annette Whiteford (CDFA)
- ICPs (CA, NV, AZ, TX)

Danish Veterinary and
Food Administration

Newcastle Disease Outbreaks in Denmark 2002

Course of Events and Status
on 10 September 2002

Danish Veterinary and
Food Administration

Summary Status

- 135 outbreaks since 15 July
  - 9 commercial flocks
  - 126 back-yard flocks
  - 4 ”primary” outbreaks – 3 virus isolations
  - 131 trade-related outbreaks
- 45 protection zones lifted until 6.Sept.

Danish Veterinary and
Food Administration

Important dates (1):

- 13 July: First clinical suspicion – no contacts
  - 15 July: serological support
  - 16 July: decision to control as ND
  - 2 August: Virological confirmation, ICPI = 1.71
- 18 July: Second clinical suspicion – cluster of contacts in Jutland
  - Clinical problems since early June
  - 19 July: serological support
  - 26 July: virological confirmation from contact, ICPI = 1.75
- 26 July: Official ND diagnosis

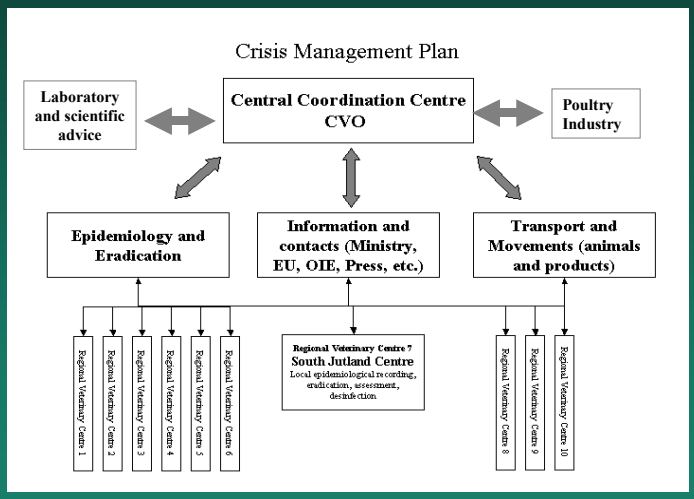
Danish Veterinary and
Food Administration

Control Strategy:

- 16. – 25. July:
  - Contacts recorded and administratively restricted
  - ND-validated suspicions treated as confirmed in Dir. 92/66, ie. slaughter, restrictions, zones, etc.
  - Trade restrictions as if confirmed outbreaks with few modifications
- Since 26. July:
  - As required in Directive 92/66 and others

Danish Veterinary and Food Administration

Following virological confirmation on 26. July:



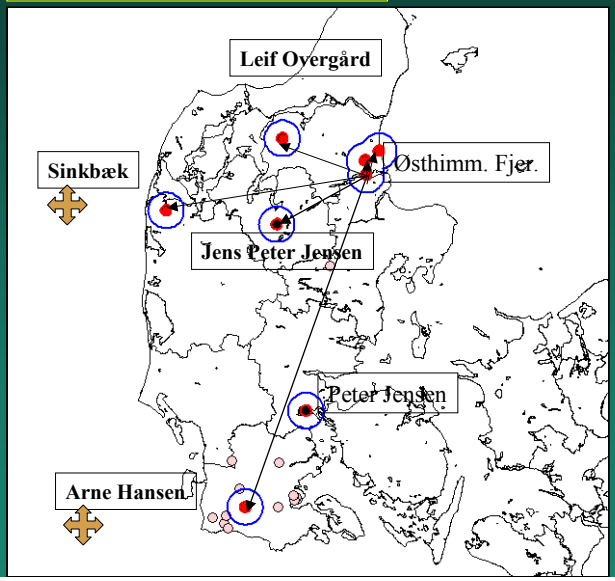
Danish Veterinary and Food Administration

Important dates (2):

- 26. July: Total export ban on live birds
- 7. August: Total export ban on hatching eggs
- 19. August: 1st. regionalisation for slaughter animals and hatching eggs
- 29. August: 2nd. regionalisation for live animals and hatching eggs
- 12. September: Proposed 3rd. regionalisation for live animals and hatching eggs

Danish Veterinary and
Food Administration

Status on 20. July 2002



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Food Administration

Diagnostic Criteria:

- Virological confirmation
- Positive serology and proven or suspected introduction of infected poultry (contact) or
- Clinical signs and positive serology in commercial flocks or
- Repeated positive serology with a titre rise in paired flock samples.

Danish Veterinary and
Food Administration

Virological Confirmation

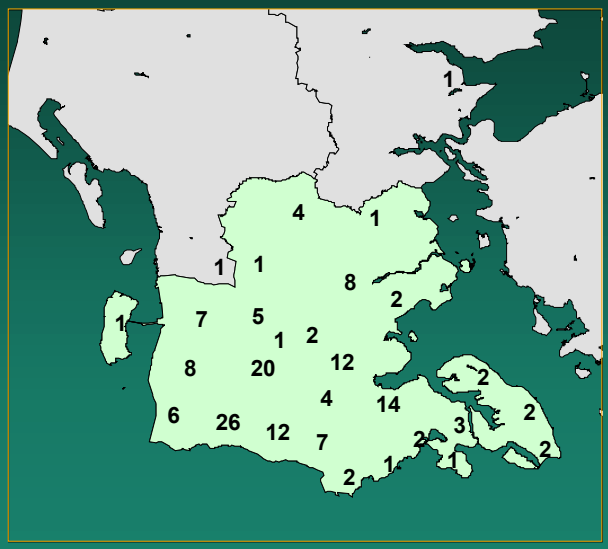
- **Danish Veterinary Institute, Aarhus:**
  - **26 July: herd # 2: APMV-1, ICPI=1.75**
  - **2 Aug.: Herd # 28: APMV-1, ICPI=1.71**
  - **4 Sept.: Herd # 127: APMV-1, ICPI=1.75**
- **EU Reference Laboratory, Weybridge:**
  - **9 aug.: Typing of two first isolates:**
    PMV-1 subtype, C1 group (velogenic)

Danish Veterinary and
Food Administration

Sønderjylland

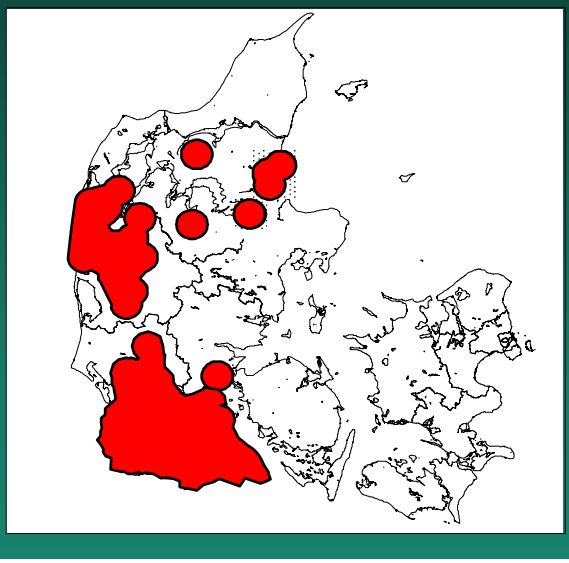
Back-yard flocks
Arne Hansen
Contacts (purchase)
Reported on
24.July at 19:00:

1 Børkop
1 Ribe
150 SDRjylland



Danish Veterinary and
Food Administration

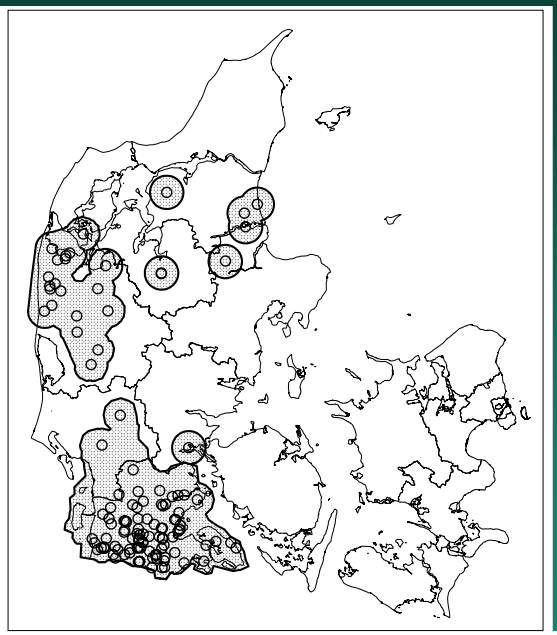
Surveillance Zones on 29. July 2002



Danish Veterinary and
Food Administration

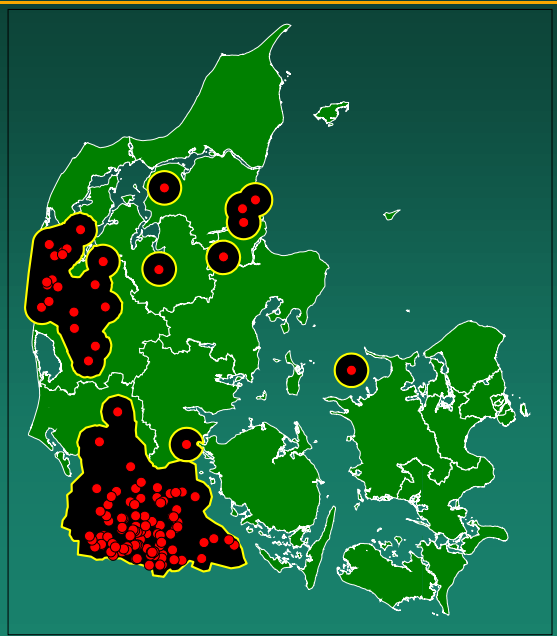
10. August 2002:

120 outbreaks and corresponding zones



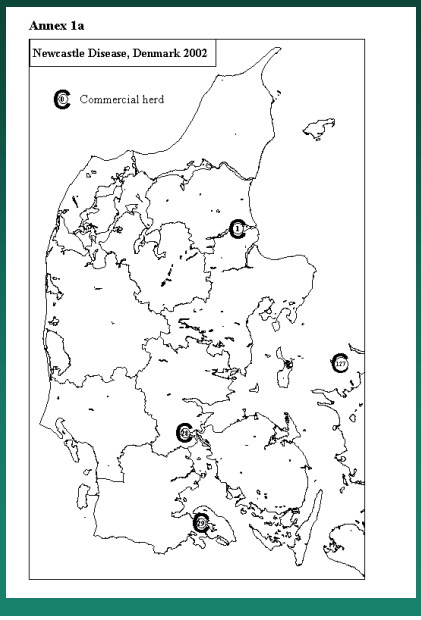
Danish Veterinary and
Food Administration

135 protection zones on 29. August



Danish Veterinary and
Food Administration

Four ”primary”, unrelated outbreaks – all within 2 km. from seashore

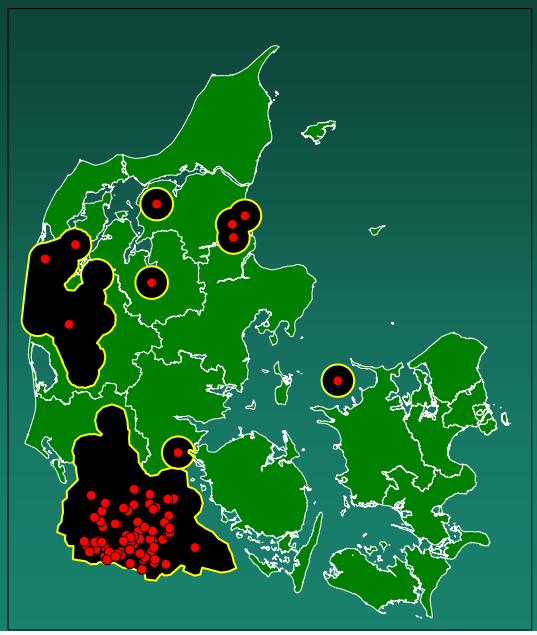


Danish Veterinary and
Food Administration

6. September:

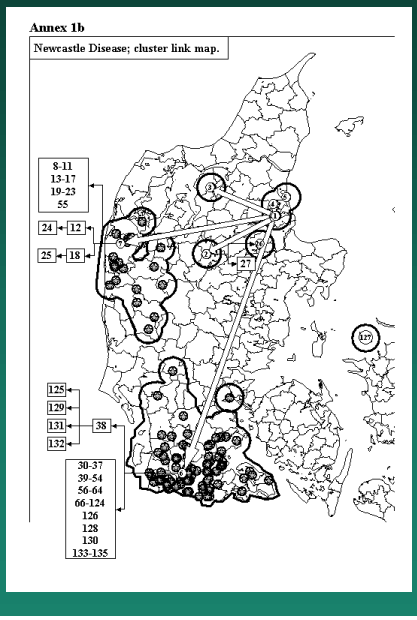
90 protection zones in place

45 protection zones lifted



Danish Veterinary and
Food Administration

Trade-related cluster of 131 outbreaks, of which 126 in back-yard flocks



Danish Veterinary and Food Administration

Status on 5. September :

Flocks:	Commercial	Back-yard
Affected poultry with ND	169.497	5.958
Holdings with ND	9	126
Eradicated holdings	9	126
Holdings with clinically affected poultry	7	6
Clinically affected poultry	164.780	804

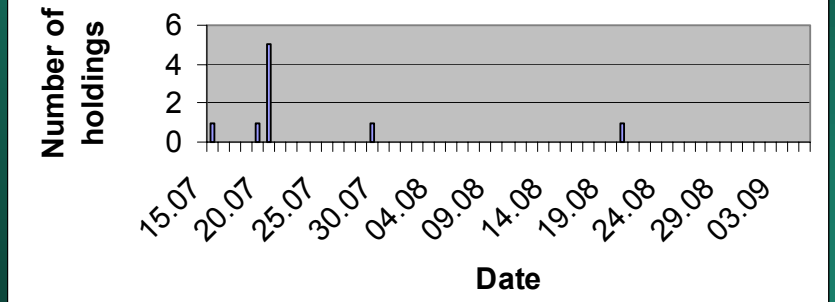
Danish Veterinary and Food Administration

Regional Distribution:

COUNTY	OUTBREAKS
– Aarhus	1
– Vejle	1
– Ribe	6
– Viborg	2
– Nordjylland	4
– Ringkøbing	20
– Sønderjylland	100
– Vestsjælland	1
TOTAL	135

Danish Veterinary and Food Administration

Commercial flocks: 9 outbreaks



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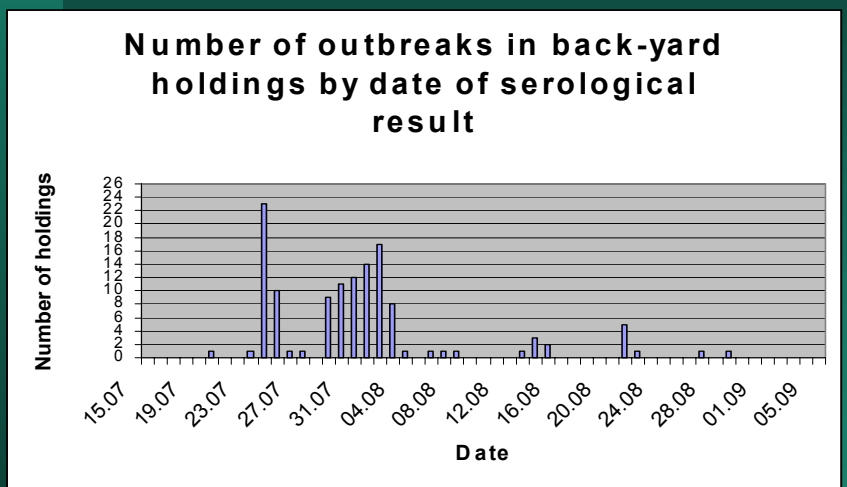
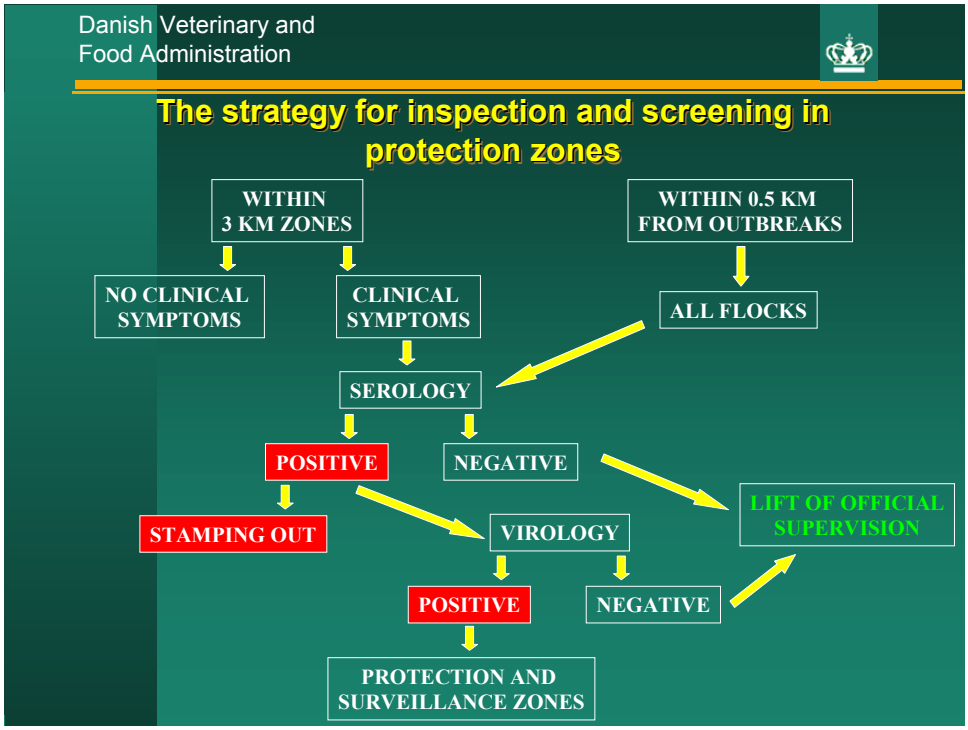
Back-yard flocks: 126 outbreaks




Danish Veterinary and
Food Administration
The strategy for inspection and screening in protection zones
WITHIN 3 KM ZONES
WITHIN 0.5 KM FROM OUTBREAKS
NO CLINICAL SYMPTOMS
CLINICAL SYMPTOMS
ALL FLOCKS
SEROLOGY
POSITIVE
NEGATIVE
LIFT OF OFFICIAL SUPERVISION
STAMPING OUT
VIROLOGY
POSITIVE
NEGATIVE
PROTECTION AND SURVEILLANCE ZONES


Danish Veterinary and
Food Administration
Screening in protection zones
Holdings:
Registrations in protection zones 3.383
Inspections in protection zones 3.278
Serologically tested 570
Serologically positive 38
Virologically confirmed 0

Danish Veterinary and
Food Administration

1st. regionalisation on 19. August

Danish Veterinary and
Food Administration

2st. regionalisation on 29. August

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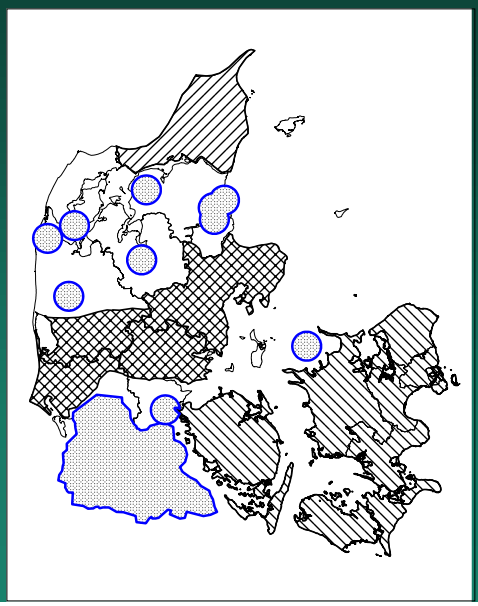
3rd. regionalisation proposed for 12. September



Danish Veterinary and
Food Administration

Legal basis:

- Order no. 921 of 10 November 1994 implementing Directive 92/66/EEC
- Order no. 598 of 16 July 2002 Gathering of poultry - e.g. at livestock shows, exhibitions, markets and via carrier pigeon flights - is prohibited throughout Denmark.
- Order no. 599 of 17 July 2002 In case of validated suspicion of ND it is possible to make a 10 kilometre surveillance zone
- 26 July 2002 A total ban on export of live poultry.
- Order no. 625 of 26 July 2002 In case of validated suspicion of ND it is possible to make a 3 kilometre protection zone
- Order no. 624 of 26 July 2002 Control measures on poultry meat origin from suspected and infected holdings
- Order no. 626 of 29 July 2002 A prohibition against trade with and transport of hobby poultry
- Order no. 630 of 31 July 2002 Control measures on poultry meat from holdings placed in protection and surveillance zones
- Order no. 638 of 2 August 2002 Testing for ND in rearing flocks (table-egg production)
- 7 August 2002 Total ban on export of hatching egg.
- Order no. 647 of 8 August 2002 Legal basis for registration of hobby flocks


Danish Veterinary and
Food Administration
Overview of countries having imposed trade restrictions due to the ND situation in Denmark
rev. 9/8 2002 13.00
Lithuania: 18/7 - Midlertidigt forbud mod indførsel af levende fjerkræ, fersk fjerkræ, fjerkræprodukter
Latvia: 29/7 - Lukket for levende fjerkræ, rugeæg, konsumæg, fjerkrækød, produkter, nonfood-produkter
Poland: 29/7: Forbud mod import og transit af levende fjerkræ, rugeæg, konsumæg, fjerkrækød og produkter heraf, konservesoprodukter samt noonfood produkter stammende fra fjerkræ. Lempelse fsa konserves og noonfood-produkter under visse betingelser(varmebehandling
Panama: 2/8 - Forbud mod import fra lande med BSE, M&K og Newcastle disease også for produkter på vej; alle produkter
Malaysia: 5/8 - Midlertidigt forbud mod import af fersk fjerkræ, produkter af fjerkræ og forarbejdede produkter heraf gældende fra. Partier på vej til Malaysia vil blive tilladt indført
Iceland : 6/8 - Midlertidigt forbud mod import af levende fjerkræ, rugeæg og produkter af fjerkræ
Swizerland: 8/8 - Importforbud for fjerkrækød og produkter heraf. Der er mulighed for import af varmebehandlede produkter med en F-værdi >3,0 eller en varmebehandling til en kernetemp. på min 70 grader Celsius

Inactivation of Avian Influenza and Newcastle Disease Viruses in Egg Products by Pasteurization

David E. Swayne
Southeast Poultry Research Laboratory
Agriculture Research Service, US Department of Agriculture, Athens, Georgia 30605

EU AI/NDV 2003

LPAI & Egg Exports

Issue: Non-tariff trade barrier when U.S. reports LPAI H5 or H7 viruses to trading partners

- Demonstrated HPAI virus in internal contents of eggs from naturally infected layers (1983-84) (Cappucci, Avian Dis 29, 1195-1200, 1985)
- LP H7N2 AI virus (Pennsylvania-1997) produced lesions in oviducts – potential for egg transmission (Zeigler, Avian Dis. 43:142-149, 1999)
- Japan has embargoed pasteurized egg products from entire USA in response to VA/WV H7N2 AI outbreak during 2002
  - Question: Will pasteurization inactivate the AI virus?
  - Gough (Vet Rec. 93:632-633, 1973) – heat inactivation vNDV (Herts)
  - King (Av Dis 35:505-514, 1991) – AIV & NDV

EU AI/NDV 2003

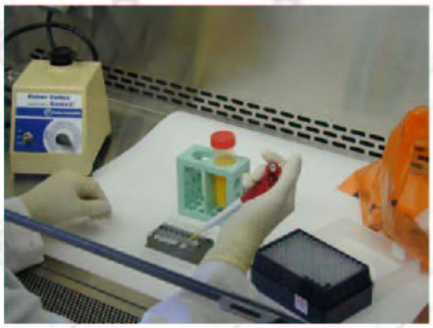
Materials and Methods

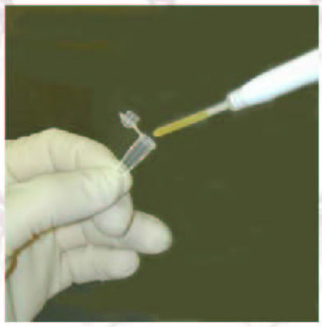
- Viruses:
  - LPAI virus: A/CK/NY/13142-5/94 (H7N2)
  - HPAI virus: A/CK/PA/1370/83 (H5N2)
  - Ulster ND virus
  - B1 vaccine virus
  - California velogenic ND virus of 2002
- Test Materials:
  - Whole egg (61C - 3.5 min)
  - Salted egg yolk* (63C - 3.5 min)
  - Dried egg white* (55C - 7d)

*Commercial products

EU AI/NDV 2003

80 µl thin-wall plastic vial





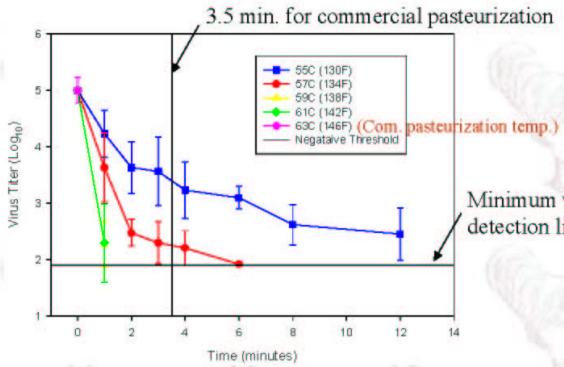
EU AI/NDV 2003

Precision thermocycler plate

EU AI/NDV 2003

Pasteurization of Egg Products

Example of Pasteurization Curves:
H7N2 LPAI virus in homogenized whole egg



3 replicates, Titer range =$10^{4.9}$ to $10^{5.1}$ EID_{50}'s/ml

USAHA2003

Pasteurization of Egg Products

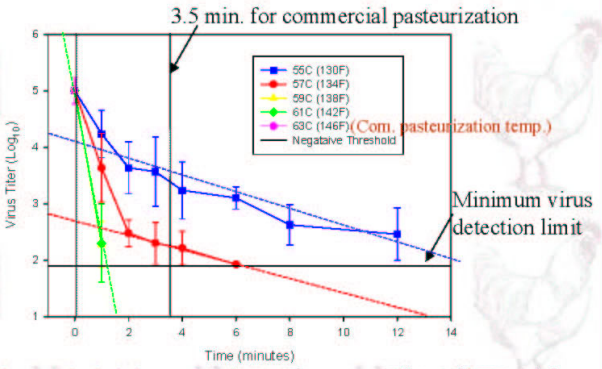
Draw straight line & calculate D_t Values

$y = -0.1251x + 3.71$
$R^2 = 0.9278$
$D_t = 8m\ (480s)$

$y = -0.1348x + 2.60$
$R^2 = 0.9922$
$D_t = 7.4m\ (445s)$

$y = -2.7333x + 7.77$
$R^2 = 1$
$D_t = 0.37\ m\ (22\ s)$

$y = -2.7567x + 7.79$
$R^2 = 1$
$D_t = 0.37\ m\ (22\ s)$



H7N2 LPAI virus in homogenized whole egg - 3 replicates, Titer range $=10^{4.9}$ to $10^{5.1}$ EID_{50}'s/ml

Data Summary

Minutes or days to totally thermal inactivate of artificially added AI and ND viruses (final concentration of $10^{4.5\text{-}6.4}$ EID_{50} virus/ml of egg product) to theoretical zero

Virus	Type of Egg Product		
	Homogenized Whole Egg (61C, 3.5min)	**10% Salted Egg Yolk (63C, 3.5min)**	**Dried Egg Whites (55C, 7d)**
A/chicken/New York/13142-5/92 (H7N2) LPAIV	2.8 min	< 1 min	3.2 days
A/chicken/Pennsylvania/1370/83 (H5N2) HPAIV	< 1 min	< 1 min	10 days
Ulster NDV - lentogen	< 1 min	< 1 min	2.6 days
B1 vaccine virus - lentogenic	< 1 min	< 1 min	2.6 days
CA END 2002 - velogenic	< 1 min	< 1 min	2.6 days

EU AI/NDV 2003

Data Summary

D_t Values – time to reduce titer by 90% (1log10 EID_{50})

Virus	Homogenized Whole Egg at 61C (sec)	10% Salted Yolk at 63C (sec)	Dried Egg White at 55C (days)
LPAI/NY/94	22	<12	0.5
HPAI/PA/83	< 12	<12	2.2
NDV/Ulster	<12	<12	0.26
NDV/B1	<12	<12	0.28
NDV/CA/02	<12	<12	0.28

Z Values – Temperature increase to reduce titer by 90% (1log10 EID_{50}) in homogenized whole egg

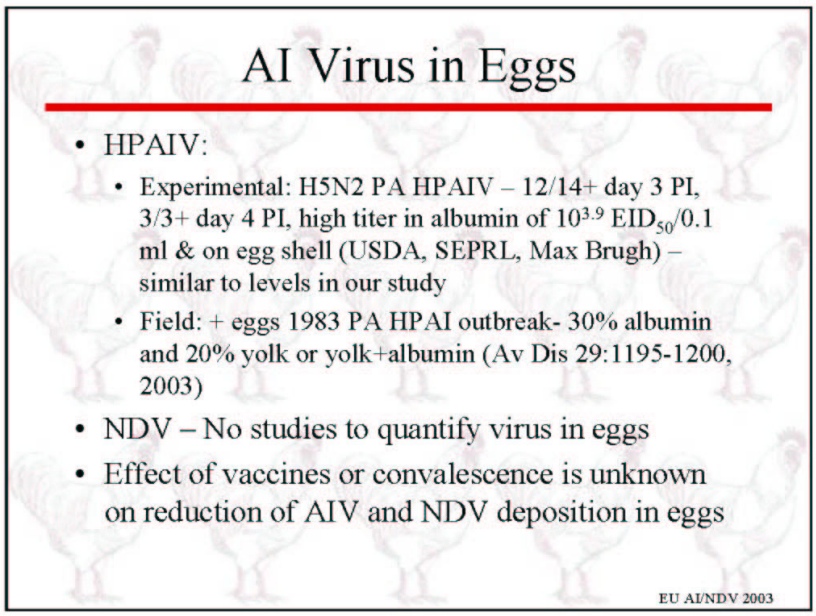
	LPAI/NY/94	HPAI/PA/93	NDV/ULSTER	NDV/B1	NDV/CA03
Z Value	1.9 C	2.5C	1.7C	1.9C	2.5C

EU AI/NDV 2003

AI Virus in Eggs

- LPAIV:
  - Experimental: H4N8 LPAIV – IN layers; 0/20+ eggs (Av Dis 38:22-32. 1994)
  - Experimental: H5N2 PA LPAIV – 0/92+ eggs laid days 1-9 PI (USDA, SERPL, Max Brugh)
  - Field: 2/120+ in 1 layer flock without mortality,1983 PA HPAI outbreak – virus "NP" in chickens (mixed infection?) (Av Dis 29:1195-1200, 2003)

EU AI/NDV 2003


AI Virus in Eggs
• HPAIV:
• Experimental: H5N2 PA HPAIV – 12/14+ day 3 PI, 3/3+ day 4 PI, high titer in albumin of $10^{3.9}$ EID_{50}/0.1 ml & on egg shell (USDA, SEPRL, Max Brugh) – similar to levels in our study
• Field: + eggs 1983 PA HPAI outbreak- 30% albumin and 20% yolk or yolk+albumin (Av Dis 29:1195-1200, 2003)
• NDV – No studies to quantify virus in eggs
• Effect of vaccines or convalescence is unknown on reduction of AIV and NDV deposition in eggs
EU AI/NDV 2003

RELATION BETWEEN TRANSMSSION OF AND IMUNE RESPONSE AGAINST NEWCASTLE DISEASE

Author: G. Koch

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Quantification of transmission:

Reproduction ratio R_0

- R_0 is defined as the average number of secondary cases caused by one infectious animal in a population of susceptible animals.

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REPRODUCTION RATIO R_0

- R_0 has a threshold value of 1
- When R_0<1 the infection chain will fade out, outbreaks remain minor.
- When R_0>1the infection will spread and the probability on a major outbreak increases the larger R_0

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DATA ANALYSIS: S-I-R model

- S (susceptible)
- I (infectious)
- R (recovered)

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STOCHASTIC SIR MODEL

S **I** **R**

S = Susceptible
I = Infectious
R = recovered
ß = parameter for rate of infection
α = parameter for rate of recovery

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Basic design of a transmission experiment

- Group of N chickens (20)
- Challenge 10 chickens I_0 = 10. This starts the chain of infections
- Place 10 chickens in contact (S_0 = 10)
- Transmission is estimated from the number of contact infections.

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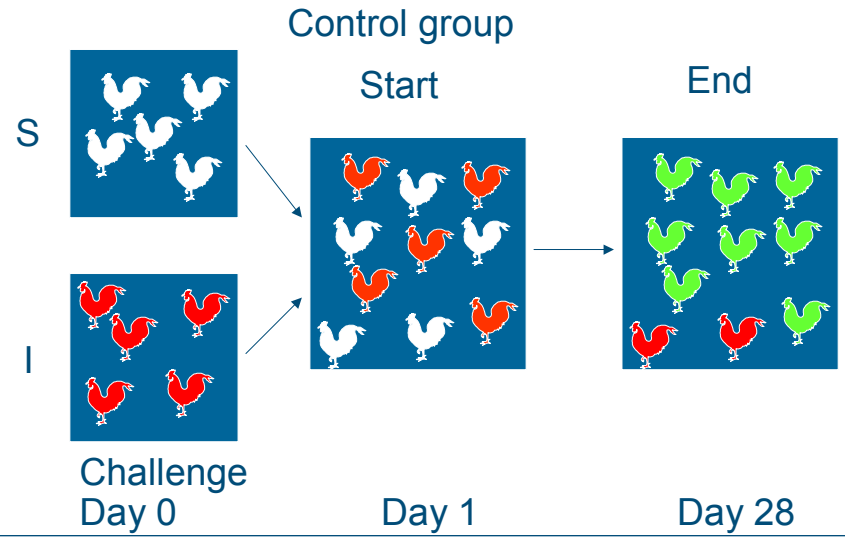
Basic design of transmission experiment



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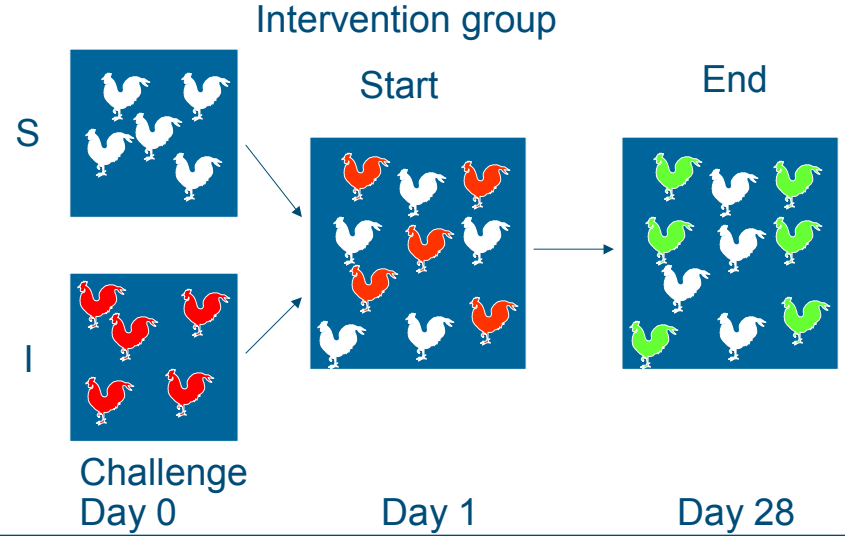
Basic design of transmission experiment



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Transmission of virulent NDV in chickens with different levels of immunity

Titre group ^{2}log	geometric mean titre¶	Mortality of		Seroconversion of		R_0 (MLE)
		Chall.§	Contact	chall.	Contact	
0-1	0,2± 0,37	0/10	0/10	6/10	5/10	1.04 (0.32-3.44)
0-8[f]	1,7± 2,41	1/10	1/10	2/10	2/10	0.80 (0.18-4.74)
3-4	2,8 ± 0,77	1/10	0/10	1/10	2/10	0.69 (0.11-7.81)
>4	5,5± 1,24	0/10	0/10	0/10	0/10	0.00 (0.00-0.89)
SPF	ND	3/5	1/5	0/5	0/5	0.46 (0.02-5.17)

¶ ^{2}log of Mean HAI titre ± standard deviation with Ulster antigen.
§ challenge with California
[f] group not selected for HAI titre

CIDC-LELYSTAD WAGENINGEN UR

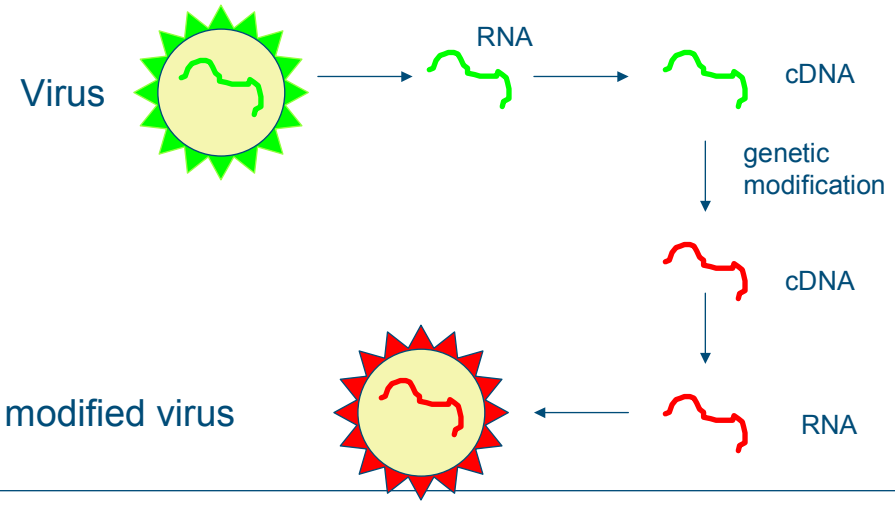
Transmission of virulent NDV in chickens with different levels of immunity

Titre group (log_2)	Mean titre¶	Mortality of		Serocon-version of		R_0 (mart.)	R_0 (MLE)¶
		chall.§	contact	chall.	contact		
0-1	0,5	0/10	1/10	10/10	10/10	N.D.	∞ (1,158-∞)
0-8[f]	1,95	0/10	3/10	9/10	6/10	N.D.	∞ (1,158-∞)
3-4	3,5	1/10	0/10	3/10	3/10	0,67	0,6 (0,1-2,4)
>4	6,2	0/10	1/10	0/10	0/9	0,182	0,2 (0,0-1,3)
0	0	0/5	0/5	5/5	5/5	N.D.	∞ (0.68-∞)

¶ ^{2}log of Mean HAI titre.
§ challenge with PMV/152608/NL/92
[f] group not selected for HAI titre

CIDC-LELYSTAD WAGENINGEN UR

Construction of an infectious clone



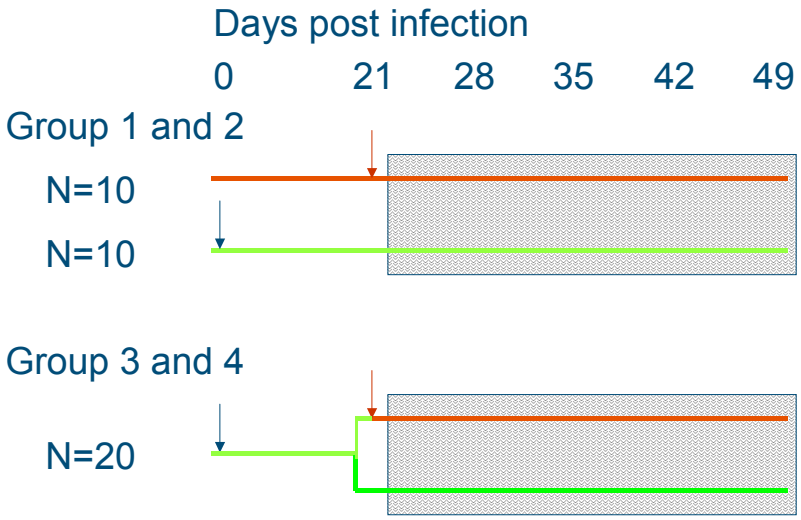
CIDC-LELYSTAD
WAGENINGEN UR

Transmission experiments using a marker vaccin

- Haemagglutinin of PMV-1 has been exchanged by the haemagglutinin of a PMV-2 strain

CIDC-LELYSTAD
WAGENINGEN UR

Experimental design



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Seroconversion and mortality after challenge.

Group	Vaccinated	Route	Nr. birds with seroconversion or dead after vaccination at day:					
			0	20	28	35	42	49
1	+	contact	0	0	0	10	10	10
	-	in/io	0	0	7	3		
2	+	contact	0	0	0	10	10	10
	-	in/io	0	0	10			
3	+	contact	0	0	0	0	0	0
	+	in/io	0	0	9[α]	9	9	9
4	+	contact	0	0	0	0	0	0
	+	in/io	0	0	7	10	10	10

[α] 1 bird died between day 20 and 28 after vaccination.

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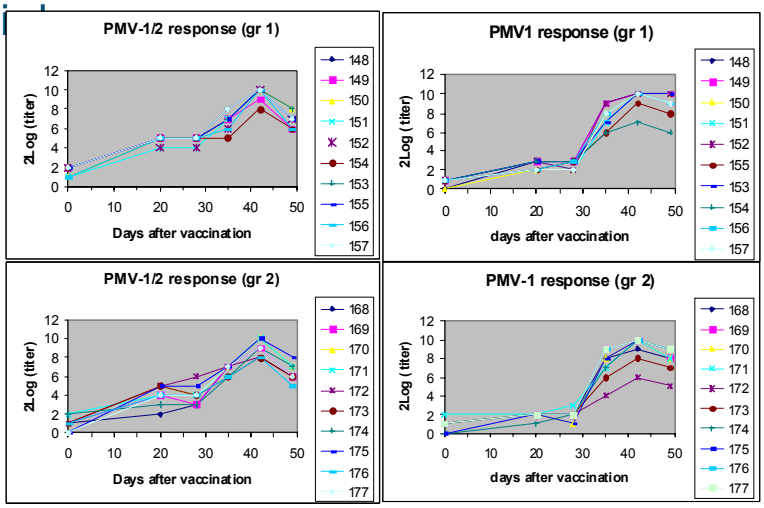
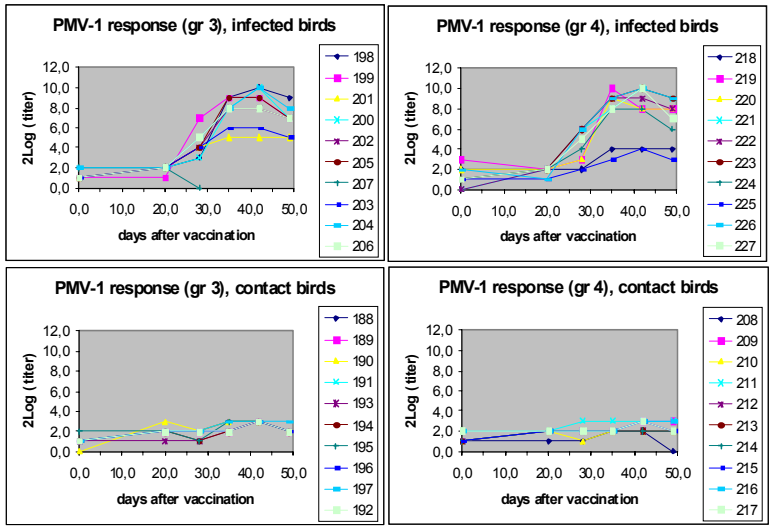
HI response after vaccination in contact bi



CIDC-LELYSTAD WAGENINGEN UR



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Conclusions

- A correlation between immunity and transmission of NDV does exist.
- Titres of about 1:8 or larger required to prevent larger outbreaks within a flock
- DIVA vaccine is in principle possible for NDV

CIDC-LELYSTAD WAGENINGEN UR

Thanks for your attention

CIDC-LELYSTAD WAGENINGEN UR

MOLECULAR EPIDEMIOLOGY OF PIGEON PPMV-1 VIRUSES

Liz Aldous, Ian Brown & Dennis Alexander

Virology Department, VLA Weybridge, Addlestone, Surrey, UK.

A variant strain of Newcastle disease virus [APMV-1] was responsible for the panzootic in pigeons and doves [both domestic and feral], which reached Europe in the early 1980s. This strain has been termed pigeon paramyxovirus type 1 [PPMV-1] and isolates have usually been identified as such by their unique reaction to a panel of monoclonal antibodies [mAbs] (Alexander *et al*., 1997), although phylogenetic studies have indicated that the isolates cluster on a separate lineage designated 4b by Aldous *et al.*, (2003), but which is synonymous with lineage VIb of Lomniczi et al, (1998). Isolates of PPMV-1 continue to be made from pigeons and doves across Europe [see Country APMV reports paper above].

In the present study a sequence of 375 nucleotides in length, which included the region encoding the cleavage activation site and signal peptide of the fusion protein gene, was determined for 181 isolates of PPMV-1 [identified by mAb reactions]. These were compared with the sequences of 44 similar isolates published on GenBank, which included 27 isolates from pigeons and 17 representatives from each sub lineage of APMV-1 (Aldous *et al.*, 2003). The resulting alignment was analysed phylogenetically using maximum likelihood and the results are presented as an unrooted radial phylogram [Fig. 1]. By phylogenetic analysis all the PPMV-1 isolates except one were placed in lineage 4b (VIb). Within this lineage there was considerable genetic heterogeneity, which appears to be predominantly influenced by the date of isolation, and to a lesser extent geographical origins of the isolates. There were two large distinguishable groups, 4bi and 4bii, which could each be divided in to three further subgroups. The earliest isolate available, PIQPI78442, isolated in 1978 in Iraq, despite being located in lineage 4b, could not be allocated to either group 4bi or 4bii. The location of isolate PIQPI78442 in the phylogram at the base of the node from which the two groups, 4bi and 4bii, divide indicates the potential role of this, or a very similar isolate as a probable progenitor virus of the PPMV-1 strain, which is in good agreement with a previous study in which it is proposed as the first isolation of PPMV-1 (Kaleta *et al*., 1985).

There is a clear temporal relationship between the strains of virus present in the two groups. The majority of the viruses in 4bi were isolated in the first half of the panzootic between the early 1980s and the early 1990s, whereas those in 4bii were generally isolated from the latter half, the early to mid 1990s onwards. The reasons for this progressive variation over the course of the panzootic are not clear. Despite this temporal separation of 4bi and 4bii viruses, there is no evidence of sequential progression through the six subgroups; commonly viruses from several of the six subgroups co-circulate in the pigeon and dove populations in a country at the same time. This is in agreement with previous studies where it has been concluded that at any one time there are a number of genetically distinct APMV-1 virus pools circulating in a host population.

REFERENCES

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Alexander, D. J., Manvell, R. J., Lowings, J. P., Frost, K. M., Collins, M. S., Russell, P. H. & Smith, J. E. (1997b). Antigenic diversity and similarities detected in avian paramyxovirus type 1 (Newcastle disease virus) isolates using monoclonal antibodies. *Avian Pathology, 26,* 399-418.

Kaleta, E.F., Alexander, D.J., & Russell, P.H. (1985). The first isolation of the avian PMV-1 virus responsible for the current panzootic in pigeons? *Avian Pathology, 14,* 553-557.

Lomniczi, B., Wehmann, E., Herczeg, J., Ballagi, Pordany A., Kaleta, EF, Werner, O., Meulemans, G., Jorgensen, PH, Mante, AP, Gielkens, AL, Capua, I. & Damoser, J. (1998). Newcastle disease outbreaks in recent years in Western Europe were caused by an old (VI) and a novel genotype (VII). *Archives of Virology, 143,* 49-64.

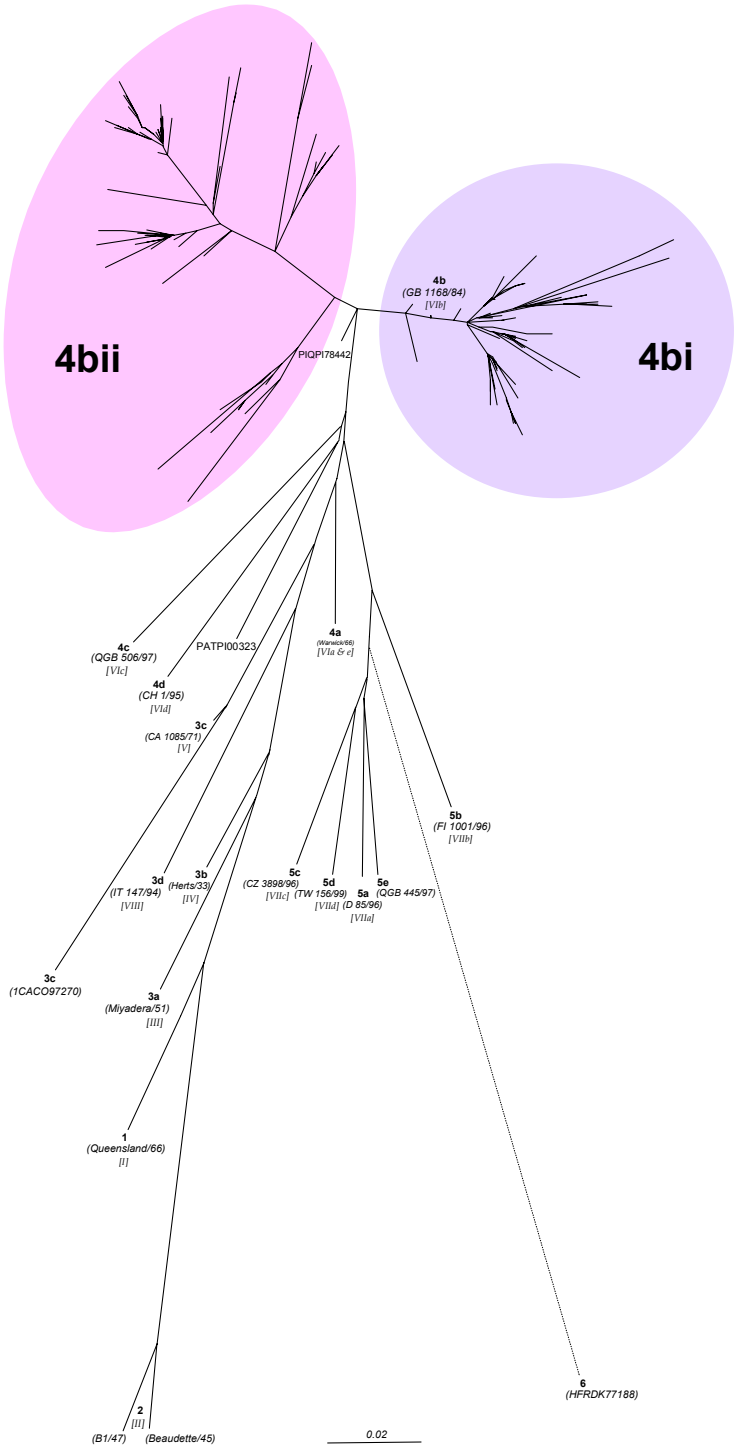

Figure 1. *Unrooted maximum likelihood radial phylogram based on nucleotide sequence data from 226 APMV-1 isolates; including 208 PPMV-1 isolates and 17 representative of the other genetic lineages (Aldous et al., 2003). The region analysed was a 375bp fragment (47-422) at the 3' end of the fusion protein gene. Branch lengths represent the predicted number of substitutions and are proportional to the differences between the isolates. The branch to isolate HFRDK77188 (group 6) is not drawn to scale; its actual branch length value is 1.66.*

COMPARATIVE TESTS FOR ANTIGEN IDENTIFICATION IN DIFFERENT NATIONAL LABORATORIES 2003

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Surrey KT15 3NB, United Kingdom.

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 and 2001. At the 8th 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 8th 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.

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	PPMV-1 pigeon/England/617/83	APMV-1
B	APMV-3 turkey/England/1087/82	APMV-3
C	A/turkey/Wisconsin/66 (H9N2)	not APMV-1, H5 or H7
D	A/turkey/Ontario/7732/66 (H5N9)	H5
E	A/turkey/England/647/77 (H7N7)	H7

RESULTS

General

Twenty-eight laboratories that had been sent samples responded by submitting results. These results are shown in Table 1. All 16 EU laboratories responded, this included additional laboratories for N. Ireland and separate influenza and Newcastle disease laboratories for Greece. While Belgium acts as both reference laboratories for Luxembourg. Laboratories from 12 non-EU states participated these were: Bulgaria, Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovak Republic, Slovenia and Switzerland.

In total 140 results were received from the 28 laboratories. The correct results were obtained on 125 [89.0%] occasions. Fifteen [11.0%] were wrong either because the laboratory failed to identify APMV-1 or H5 antigens [all H7 results were correct], or, more usually, because the APMV-3 virus was identified as APMV-1 or the H9N2 antigen as H5 or on one occasion H6.

Of the 28 participating laboratories, 15 fully identified all HA antigens and three others had acceptable results. Six laboratories had one unacceptable result and 4 had more than one unacceptable result.

Results by antigen

ANTIGEN A – PPMV-1/pigeon/England/617/83 – correct result APMV-1

Thirteen laboratories identified this antigen as PPMV-1 using the specific monoclonal antibody available from the CRL. A further 14 laboratories also achieved the correct result, identifying the virus as APMV-1. One laboratory [5] failed to identify the antigen as APMV-1.

ANTIGEN B – virus APMV-3/turkey/England/1087/82 – correct result APMV-3

The cross reaction between APMV-1 and APMV-3 viruses in HI tests is well known and well-documented. It is important that national laboratories are aware of this and are capable of distinguishing between the two. In addition it was one of the recommendations made in the proceedings of the 5th Annual Meeting [Alexander and Manvell, 1999] and re-emphasised at subsequent meetings that all laboratories should hold APMV-3 antiserum to enable identification.

Twenty-two of the laboratories identified the antigen as APMV-3. Two laboratories reported the not wholly correct result of APMV, but not APMV-1. One laboratory [10] identified the antigen incorrectly as APMV-1 and three [3, 18, 27] as APMV-1.

ANTIGEN C – virus A/turkey/Wisconsin/1/66 (H9N2) – correct result not APMV-1, H5 or H7

This antigen was chosen because of the current widespread prevalence of H9N2 viruses throughout the World. This antigen caused the greatest problems of the five. Sixteen laboratories identified the presence of H9 influenza and 12 of these laboratories correctly stated that the neuraminidase was N2. A further 5 laboratories gave an acceptable correct result stating that the virus was not APMV-1, H5 or H7. Seven laboratories failed to identify the antigen correctly. One laboratory identified the virus as H6N3 [11] and one [14] merely stated 'not APMV-1', which could be a omission in the statement rather than an inability to determine that it was not H5 or H7 as well. However, five laboratories [3, 4, 5, 23 & 24] obtained wholly incorrect results, identifying the antigen as H5.

The identification of this virus as H5 was probably a result of cross reaction of the neuraminidase where antiserum to H5N2 had been used. It is important that laboratories recognise the possibility that neuraminidase antibodies may affect HI tests. To some extent this can be overcome if the recommendation that more than one H5 and H7 antisera prepared against viruses with different neuraminidases are always used for the identification of viruses and antigens.

ANTIGEN D – virus A/turkey/Ontario/7732/66 (H5N9) – correct result H5

Twenty-seven of the laboratories identified this antigen correctly as H5 subtype and five gave the neuraminidase correctly (two laboratories gave the neuraminidase as N2). Only one laboratory [27] produced an incorrect result stating the virus was not 'NDV, H5 or H7'.

ANTIGEN E – virus A/turkey/England/647/77 (H7N7) – correct result H7

This was the only antigen for which all 28 laboratories gave the correct result. Three laboratories gave the additional correct information that the neuraminidase was N7, although one laboratory incorrectly identified it as N1 and another as N3

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. Of the laboratories taking part in 2003 all had taken part in 2002. The comparative results for the two years were:

Number that:-

	2002	2003
Satisfactorily identified all antigens:	16	18
Had one unacceptable result	7	6
Had more than one wrong	5	4

In fact 8 laboratories showed an improvement; 12 were the same with all results correct; one was the same with one incorrect result and 7 laboratories obtained worse results than 2002. No country fell into any other possible category.

Despite the slight overall improvement of correct results of 89% correct in 2003 compared to 87% in 2002, there still appears to be an unacceptable level of incorrect results in this simple but important area of the work of the National Laboratories. It is recommended that another antigen identification comparison test is done in 2004.

ACKNOWLEDGEMENT

The CRL apologises to the NRL Norway, who for some reason never received the antigens for testing.

Table 1. Results of comparative antigen identification tests

	A	B	C	D	E
CRL	**PPMV-1**	**APMV-3(ty)**	**H9N2**	**H5N9**	**H7N7**
1	PPMV-1	APMV-3	H9N2	H5N9	H7N7*
2	APMV-1	APMV-3	H9N2	H5	H7**N1**
3	APMV-1	**APMV-1**	**H5N2**	H5**N2**	H7**N1**
4	APMV-1	APMV-3	**H5**	H5	H7
5	**APMV**	**APMV**	**H5**	H5	H7
6	APMV-1	APMV-3	H9	H5	H7
7	APMV-1	APMV-3	Not NDV, H5 or H7	H5	H7
8	PPMV-1	APMV-3(ty)	H9N2	H5N9	H7
9	PPMV-1	APMV-3	Not PMV, H5 or H7, H9N2?	H5	H7
10	PPMV-1	**APMV-7**	H9N2	H5	H7
11	PPMV-1	APMV-3	**H6N3?**	H5N9	H7N7
12	PPMV-1	APMV-3	H9N2	H5N9	H7
13	APMV-1	APMV-3	H9	H5	H7
14	APMV-1	**APMV not PPMV-1, LS or U2C**	**Not APMV 1**	H5	H7
15	APMV-1	APMV-3	H9N2	H5	H7
16	PPMV-1	APMV-3	H9N2	H5N9	H7N7
17	PPMV-1	APMV-3	H9 N2?	H5 N9?	H7
18	APMV-1	**APMV-1**	Not NDV, H5 or H7	H5	H7
19	PPMV-1	APMV-3	H9N2	H5	H7
20	PPMV-1	APMV-3	AI not H5 or H7	H5	H7
21	PPMV-1	APMV-3	H9N2	H5	H7
22	PPMV-1	APMV-3	H9	H5	H7
23	APMV-1	APMV-3	H5	H5	H7
24	APMV-1	APMV-3	H5	H5	H7
25	APMV-1	APMV-3	H9	H5	H7
26	PPMV-1	APMV-3	H9N2	H5	H7
27	APMV-1	APMV-1	Not NDV, H5 or H7	**Not NDV, H5 or H7**	H7
28	APMV-1	APMV-3	H9N2	H5**N2**?	H7**N3**

* LPAI - EIPKGR*GLFG

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2004

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 2004

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. 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.

10. Finalise work carried out in respect to the surveys in poultry and wild birds during 2003 and revise survey guidelines for surveys to be carried out during 2004.

11. Carry out work in relation with surveys for avian influenza to be implemented by Member States during 2004.

12. 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, 2004

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 2004

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.

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

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

9. 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.

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