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

HELD IN PADOVA, ITALY
19th - 21st JUNE 2002

Edited by Dennis J. Alexander

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PARTICIPANTS

EU NATIONAL LABORATORIES:

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THE NETHERLANDS:	Dr Guus Koch
	Dr Ron Fouchier
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CZECH REPUBLIC:	Dr Jitka Hornickova
ROMANIA:	Dr Gratiela Brad
SLOVENIA:	Dr Olga Zorman Rojs
BULGARIA:	Prof Georgi Hadjiev

	Dr Gabriela Goujgoulova
ESTONIA:	Dr Ants Jauram

GUEST

HONG KONG:	Prof Ken Shortridge

PROGRAMME

Wednesday 19 June (voluntary for "early arrivals")

Pratical session

12.00-16.00	N1-N3 discriminatory test Histopathology and immunohistochemistry Application of GIS to the HPAI epidemic

Thursday 20 June

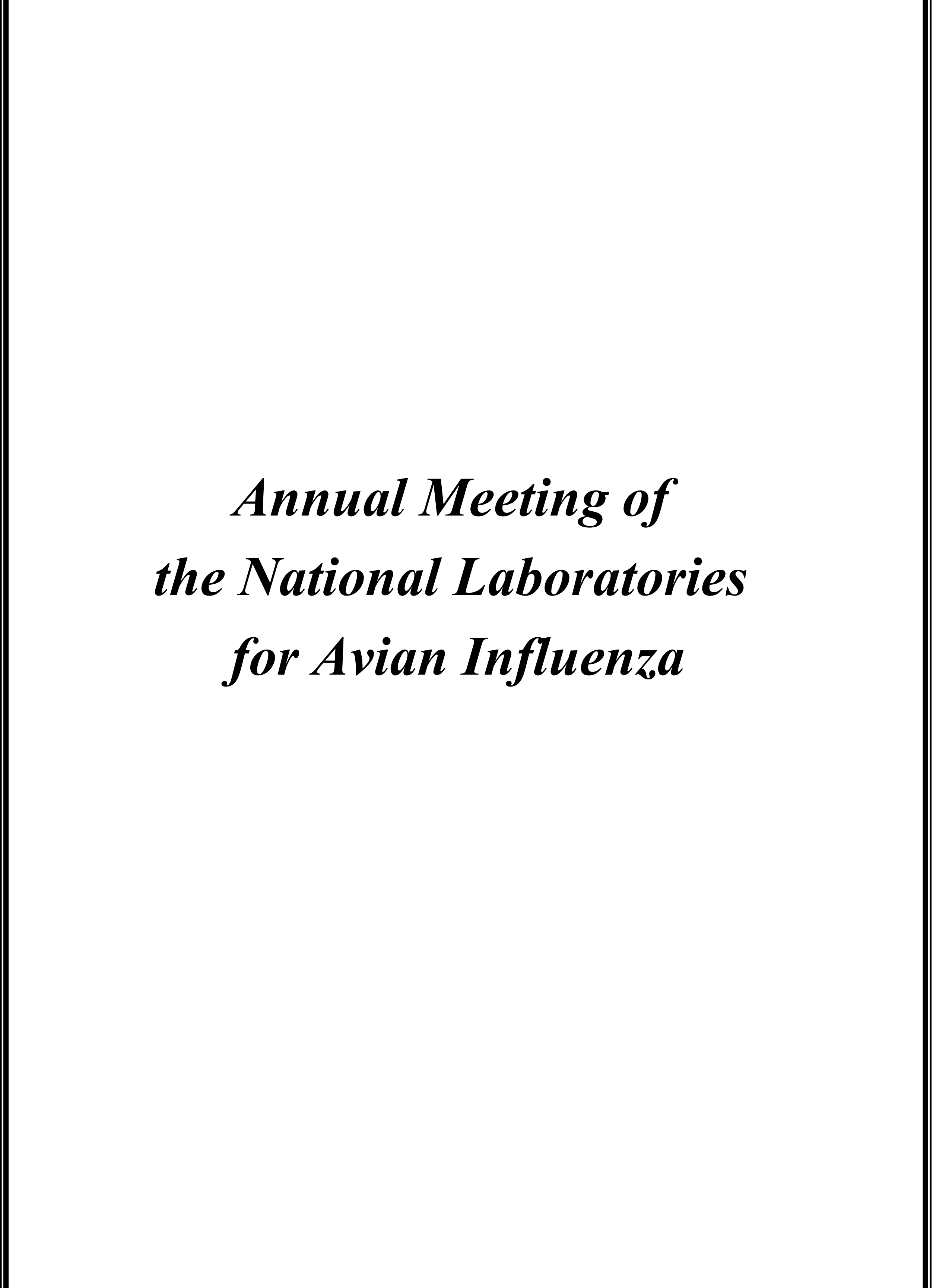
Annual meeting of the National Laboratories for avian influenza (AI)

09.15 - 09.30	Welcome
09.30 -10.00	Report from the EU Reference Laboratory ***Dennis Alexander***
10.00 -10.30	Country reports on AI based on questionnaires ***Dennis Alexander***
10.30 -10.50	*Coffee*
10.50 -11.50	Original contributions on AI
10.50 -11.05	DNA vaccination against LP H7 using coadministration of plasmids encoding HA and M1 or HA and NP ***Veronique Jestin***
11.05 -11.20	Comparative studies of the pathogenicity of AIV isolates in chickens and turkeys ***Ortrud Werner***
11.20 -11.35	Strategies for eradication of avian influenza in Italy ***Ilaria Capua***
11.35 -11.50	Influenza surveillance in wild birds in Northern Europe ***Ron Fouchier***
11.50 -12.20	Surveillance for AI in poultry and wild birds ***Ian Brown***
12.20 -12.50	Discussion
12.50 -14.00	*Lunch*
14.00 -15.00	AI situation in Hong Kong ***Prof Ken Shortridge***
15.00 -15.15	Discussion
15.15 -15.45	*Coffee*
15.45 -16.15	AI situation worldwide ***Ruth Manvell***
16.15 -16.45	Main issues emerging from the AI symposium in Athens ***Guus Koch***
16.45 -17.00	Discussion

Friday 21 June

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

09.15 -09.45	Country reports on ND based on questionnaires ***Dennis Alexander***
09.45 -10.15	Report from the European Commission ***Maria Pittman***
10.15 -10.45	*Coffee*
10.45 -11.15	Interlaboratory comparative tests ***Dennis Alexander***
11.15 -11.30	Original contribution: pPMV1 distribution in organs using a NP-based RT-PCR ***Veronique Jestin***
11.30 -12.30	Discussion of laboratory issues led by ***Ilaria Capua***
12.30 -14.00	*Lunch*
14.00 -14.30	ND situation worldwide ***Ruth Manvell***
14.30 -14.45	Work plan of the Community reference laboratory for 2003
14.45 -15.00	Discussion and close



Annual Meeting of the National Laboratories for Avian Influenza

TECHNICAL REPORT OF THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2001

Dennis J. Alexander and Ruth J. Manvell

Community Reference Laboratory for Avian Influenza
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I. LEGAL FUNCTIONS AND DUTIES

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

II. OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2001

(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 N° L 156, p. 76) as amended by Decision 96/619/EC (OJ N° L 276, p. 18). This will 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 over the past 14 years 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***
605	*284*	*266*	*305*	*357*	*704*	*316*

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**: 67 %*

WORK DONE: The viruses submitted in 2001 were characterised as shown in Table 2.

Table 2: Identification of viruses submitted to the CRL in 2001

Virus identification	Number
Paramyxoviruses	***176***
Influenza A viruses	***58***
H3N2	1
H3N8	8
H6N1	1
H6N2	1
H7N1	12
H7N3	7
H9N2	19
H10N1	2
H10N7	6
H11N9	1
Others	***82***
Reovirus	16
Herpesvirus	12
Flavivirus	2
Circovirus	1
Untyped	34
Virus not viable	17

In addition to conventional typing of the viruses a total of 30 viruses was subjected to nucleotide sequencing and the amino acids at the haemagglutinin cleavage site were deduced. Of these 3 had multiple basic amino acids at the cleavage site and were therefore HPAI viruses, 27 had amino acid motifs consistent with viruses of low pathogenicity.

Estimated actual resources: 68%

(2) Maintain virus repository and distribute viruses from it 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**: 10 %*

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:

ANTISERA:- H3 – 6 x 0.5ml, H4 – 4 x 0.5ml, H5 – 26 x 0.5ml, H7 – 25 x 0.5ml, H9 – 10 x 0.5ml, H11 – 2 x 0.5ml, AGID+ve – 25 x 0.5ml.

ANTIGENS:- AGID [Beard] antigen – 29 x 1ml, H3 – 5 x 1ml, H5 – 16 x 1ml, H7 11 x 1ml, H9 – 7 x 1ml.

Estimated actual % resources: 10%

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	30	8	150	8
H7	45	8	100	8

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

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

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

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

***% Resources**: 8 %*

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

Estimated actual % resources**: **8%

(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 will be 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) Support 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 to be held in Uppsala Sweden in April 2001.

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.

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

(8) Collecting and editing of material for a report covering the annual meeting of National Avian Influenza Laboratories to be held in Uppsala, April, 2001.

***Work Plan**: Receive and collate submissions edit and produce report of proceedings by end of 2001.*

***% Resources**: 2 %*

WORK DONE: Collation of the material and some editing has been undertaken, but at the end of 2001 the Proceedings had not been completed and published.

***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.

***% Resources**: 1 %*

WORK DONE: This aspect was reviewed continuously throughout 2001 and staff of the CRL were involved in the WHO Influenza Network specifically reviewing this possibility.

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

(10) 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 2001 relating to the work of the CRL on avian influenza:

RELEVANT PUBLICATIONS IN 2001

1. ALEXANDER, D.J. & GOUGH, R.E. (2001). Avian influenza in turkeys – A review. Proceedings of the 3rd International Symposium on Turkey Diseases Berlin 14-17th June 2000. Free University of Berlin. pp 119-131.
2. DONATELLI, I., CAMPITELLI, L., DI TRANI, L., PUZELLI, S., SELLI, L., FIORETTI, A., ALEXANDER, D.J., TOLLIS, M., KRAUSS, S., & WEBSTER, R.G. (2001). Characterisation of H5N2 influenza viruses from Italian poultry. Journal of General Virology 82, 623-630.
3. BANKS, J., SPEIDEL, E.S. MOORE, E., PLOWRIGHT, L., PICCIRILLO, A., CAPUA, I., CORDIOLI, P., FIORETTI, A. & ALEXANDER D.J. (2001). Changes in the

haemagglutinin and the neuraminidase genes prior to the emergence of highly pathogenic H7N1 avian influenza viruses in Italy. Archives of Virology, 146, 963-973.
4. ALEXANDER, D.J. (2000). Highly pathogenic avian influenza (fowl plague). Chapter 2.1.14. OIE Manual of Standards for diagnostic tests and vaccines. OIE : Paris pp 212-220
5. CAPUA, I., MARANGON, S., MUTINELLI, F. & ALEXANDER, D.J. (2001). Laboratory diagnosis during the 1999-2000 avian influenza epidemics in Italy. Proceedings of the World Association of Veterinary Laboratory Diagnosticians X International Symposium, Parma, Italy July 2001 pp99-100.
6. ALEXANDER, D.J. (2001) Orthomyxoviridae – avian influenza. In Poultry diseases. F. Jordan. M. Pattison, D. Alexander & T. Faragher [eds]. W.B. Saunders, London pp 281-290.
7. ALEXANDER, D.J. (2001) Ecology of avian influenza in domestic birds. Proceedings of the International Symposium on Emergence and Control of Zoonotic Ortho- and Paramyxovirus Diseases, B. Dodet & M. Vicari (eds). Merieux Foundation Veyrier-du-lac France. pp 25-34.
8. ALEXANDER, D.J. & CAPUA, I. (2001). Avian influenza and human health. Proceeding of the 3rd International Congress on Emerging Zoones Noordwijkerhout, Holland October, 2001 p37.
9. Manvell, R.J., Magnino, S., Wernery,U. & Moreno-Martin, A. Two recent isolations of avian influenza virus subtype H7 from falcons. Proceedings of the 6th European AAV-DVG Conference, Munich, Germany. March 7 - 10, 2001 p 212-214.
10. Wernery, U., Molnar, L., Manvell, R.J., Kinne, J. & Joseph, S. (2001). Influenza virus infections in houbara bustards (*Chlamydotis undulata macqueenii*) in the United Arab Emirates. Proceedings of the 6th European AAV-DVG Conference, Munich, Germany. March 7 - 10, 2001 p271-276.

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

COUNTRY REPORTS ON AVIAN INFLUENZA FOR 2001 BASED ON RESPONSES TO THE QUESTIONNAIRE

Dennis J. Alexander and Ruth J. Manvell

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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:

***

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*

If there were a large number of outbreaks i.e. in Italy give numbers of isolates with same properties and ranges of IVPI.

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

***

A total of 29 questionnaires was sent to different laboratories. Responses were received for 14 laboratories of EU countries: Italy, Portugal, Spain, Greece, Ireland, UK, Northern Ireland, Denmark, Finland, France, The Netherlands, Sweden, Germany and Belgium, and 10 from non-EU countries: Slovenia, Norway, Cyprus, Estonia, Bulgaria, Poland, Slovak Republic, Latvia, Lithuania and Switzerland. The responses are summarised in the following pages.

BELGIUM/LUXEMBOURG

Samples tested

Type of bird	Sample	Method	Number
pigeons	tissues	eggs	24
	tissues	cell culture	24
chickens	tissues	eggs	47
	tissues	cell culture	47
psittacines	tissues	eggs	36
	tissues	cell culture	36
other poultry	tissues	eggs	6
	tissues	cell culture	6
small birds	tissues	eggs	55
	tissues	cell culture	50

Influenza viruses isolated

None.

BULGARIA

Serological monitoring for avian influenza antibodies

Type	flocks	sera	method	flocks +ve	sera +ve*
ducks	14	310	ELISA	7 [50%]	22 [7%]
pheasants	4	97	ELISA	4 [100%]	27 [28%]
partridges	1	13	ELISA	1 [100%]	2 [15%]
Total	20	450		12 [60%]	51 [11%]

*positive sera were not H5 or H7

CYPRUS

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
imported parrots	tissues	2

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
ostriches	50	AGID	negative

DENMARK

Samples tested by inoculation into eggs

Avian species	Number of submissions	Number of birds	Number of samples
Birds in captivity:			
Tissue samples:			
partridges	6	32	106
pigeons	33	44	150
pheasants	64	178	514
geese	1	2	4
chickens	40	80	258
turkeys	8	23	98
ostriches	7	12	45
parrots	53	58	223
other pet birds	9	16	23
birds from zoos	25	30	98
Free-living birds:			
Tissue samples:			
birds of prey	2	3	3
waterfowls	4	15	60
other birds	9	12	48
Cloacal samples:			
waterfowl	21	297	297
cormorants	11	170	170
other birds	4	58	58
Total:	297	1030	2164

Influenza viruses isolated.

None.

ESTONIA

Samples tested

Type of bird	Sample	Method	Number
Chickens	tissues	eggs	3

Influenza viruses isolated

None.

FINLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
poultry	tissues	8
	faeces	3
pet birds	cloacal swabs	18
	faeces	8
wild birds	tissues	12
	faeces	3

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies.

216 sera from imported chickens and turkeys were examined – all were negative.

FRANCE

Samples tested by inoculation into eggs:

Birds	Samples	Number
broilers	tissues cloacal and tracheal swabs	7 20
layers	tissues	2
meat turkeys	tissues cloacal and tracheal swabs	9 35
turkey breeders	cloacal and tracheal swabs	60
ducks	tissues cloacal swabs	1 20
backyard chickens	tissues cloacal and tracheal swabs	6 10
pigeons	tissues cloacal swabs	25 5
aquatic wild birds	cloacal swabs	373
experimental sentinel ducks*	cloacal swabs	160
doves	tissues	2
budgerigar	tissues	3
blackbirds	tissues cloacal and tracheal swabs	2 10

*sharing the conditions of wild birds and monitored every two weeks during the winter of 2000-2001

Influenza viruses isolated

Bird	subtype	IVPI	HA0 cleavage site	conclusion
meat turkeys (x2)	H6	0.0	nd	LPAI
experimental sentinel ducks*(x2)	H6	nd	nd	LPAI (to be checked)

Serological monitoring for avian influenza antibodies

Type of bird	No. sera	test	sera +ve
poultry nr H6 cases	420	HI	5.5% to H6
meat turkeys	4170	AGP	1.2% H6 & H?*
sentinel ducks	160	AGP	3.1% H6

*not H5 or H7

GERMANY

Virus isolation

Species	Number	Samples	Inoculation in eggs or eggs and cell culture	AIV isolates
chickens	986	tissue	986	6
broilers	21	tissue	21	0
turkeys	60	tissue	56	1
ducks	85	tissue	85	0
geese	37	tissue	37	0
ostriches	10	tissue	10	0
pigeons	250	tissue	250	1
ornamental chickens	7	tissue	7	0
pheasants,quails, peacocks, other	27	tissue	27	0
parakeets/parrots	111	tissue	111	0
exotic birds	173	tissue	173	0
zoo birds	9	tissue	9	0
crows/ravens	4	tissue	4	0
birds of prey/owls	8	tissue	8	0
wood/black grouses	4	tissue	4	0
small wild birds	22	tissue	22	0
wild water birds	6	tissue	6	0

Total 1820

AIV Isolates

Birds	Number of Isolates	Subtype
turkeys	1*	H7N7
chickens	6*	H7N7
pigeon	1	??

* isolates from the same small free-range mixed poultry flock

Characterization of AIV isolates

Bird	subtype	IVPI	HA0 cleavage site	Conclusion
turkey	H7N7	0.03	NVPEIP**KGR***GLFG	LPAIV
chicken	H7N7	0.00	NVPEIP**KGR***GLFG	LPAIV
pigeon	?	0.00		LPAIV

Serological surveillance for AIV in Germany in 2001

Surveillance for all AIV subtypes

10 samples per flock at slaughter
ELISA, AGID or HI (Subtypes 1-13)
Positive samples were subtyped by HI test

Species	Number of samples	Test			Positive samples	Subtype
		ELISA	**AGID**	**HI**		
chickens	13 669	7183	6486		0	
broilers	1 224	1224			0	
turkeys	22 414	20938	1476		375	2 x H6 373 x H1
ducks	95			95	0	
geese	109			109	30	30 x H6
pigeons	12			12	0	

Surveillance for AIV Subtype H7 only
(Monitoring after isolation of LPAIV H7N7 from a small free-range mixed poultry flock)

Species	Number of samples	positive
chickens	130	6
broilers	33	0
turkeys	8	0
ducks	7	0
geese	3	0
pheasants	2	0
pigeons	235	0
cormorants	24	0

GREECE

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues cloacal swabs	34 88
broiler breeders	cloacal swabs	23
layers	tissues cloacal swabs	25 107

Influenza viruses isolated

None

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
broilers imported from Italy	2425	AGID	negative
layers, breeders and turkeys	574	AGID	negative

IRELAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	tissues	16
turkeys	tissues	3
pheasants	tissues	5
geese	tissues	3
ducks	tissues	11
penguins	tissues	2
parrot	tissues	1
pigeon	tissues	1
swans	tissues	3
not stated	tissues	1

Influenza viruses isolated

None.

ITALY

Samples tested in eggs:

Type of bird	Sample	Number
broiler breeders	tissues pools of cloacal swabs pools of tracheal swabs	2 32 4
layer breeders	tissues pools of cloacal swabs	2 4
broilers	tissues pools of cloacal swabs pools of tracheal swabs	1 10 11
layers	tissues pools of cloacal swabs	9 48
rural chickens	tissues	7
turkey breeders	tissues pools of cloacal swabs pools of tracheal swabs	5 3 20
meat turkeys	tissues pools of cloacal swabs	39 7
pheasants	tissues pools of cloacal swabs pools of tracheal swabs	2 4 27
quail	tissues	1
guinea fowl	tissues	2
ostriches	pools of cloacal swabs	11
ducks	tissues pools of cloacal swabs	2 82
pigeons	tissues pools of cloacal swabs	2 3
collared doves	tissues	3
swans	tissues	5
wild birds	tissues	10

Influenza viruses isolated

24 avian influenza viruses of H7N1 subtype were isolated and characterised:

Birds	subtype	IVPI	HA0 cleavage site	Result
layer	H7N1	n.d.	PEIPKGR*GLF	1 x LPAI
meat turkey	H7N1	n.d.	PEIPKGR*GLF	23 x LPAI

LATVIA

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
poultry	561	?	negative

LITHUANIA

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
poultry [24 farms]	807	ELISA	negative

THE NETHERLANDS

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
pigeons	tissues	8
backyard poultry	tissues cloacal swabs	7 6
ducks	tissues cloacal swabs	1 1
gull	tissues	1
oyster catchers	tissues	4
crows	tissues	4
jackdaw	tissues	4
starling	tissues	1
sparrow	tissues	2
others	cloacal swabs	10

Influenza viruses isolated

None.

NORWAY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
layers	cloacal swabs	35

Influenza viruses isolated

None.

POLAND

Samples tested

Type of birds	Sample	Method	Number
Chickens	tissues	eggs/cell culture	16
Turkeys	tissues	eggs/cell culture	4
Pigeons	tissues	eggs	2
Ostriches	tissues	eggs	1

Influenza viruses isolated:

None

Serological monitoring for AI antibodies in 2001 (research project of NVRI)

Type of birds	No. flocks tested	No. sera tested	ELISA		AGP		HI (H5/H7)	
			No. sera tested	No. flock/sera positive	No. sera tested	No. sera positive	No. sera tested	No. sera positive
broiler breeders	25	270	270	3/3	30	0	30	0
layers	17	212	212	2/2	20	0	20	0
broilers	29	326	326	0	0	-	0	-
turkey breeders	10	102	102	1/1	10	0	10	0
meat turkey	23	245	245	1/1	10	0	10	0
geese breeders	21	210	0	-	210	0	210	0
meat geese	18	180	0	-	180	0	180	0
ducks breeders	5	50	0	-	50	0	50	0
meat ducks	14	140	0	-	140	0	140	0
pigeons	10	100	0	-	100	0	100	0
pheasants	1	10	0	-	10	0	10	0
guinea fowls	1	10	0	-	10	0	10	0

PORTUGAL

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	3
chicken	tissues	1
ducks	tissues	4
exotic birds	tissues faeces	17 7
pigeons	tissues	4
wild birds	tissues	1

Influenza viruses isolated

2 x H3N8 from exotic birds

Influenza viruses characterisation

Birds	Subtype	IVPI	HA0 cleavage site	Result
exotic	2 x H3N8	0.0	not done	2 x LPAI

Serological monitoring for avian influenza antibodies

None.

SLOVAK REPUBLIC

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
broilers and breeders	230 104	ELISA AGID	negative negative
other poultry	6	AGID	negative

SLOVENIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissues	10
broiler breeders	tissues	2
layers	tissues	4
pigeons	tissues cloacal swabs	2 4

Influenza viruses isolated

None

SPAIN

Samples tested by inoculation into eggs:

Birds	Sample	Number	Result
Barn owl	cloacal swabs	1	negative
Booted eagle	cloacal swabs	1	negative
Budgerigar	cloacal swabs	1	negative
Buzzard	cloacal swabs	3	negative
Canary	cloacal swabs	295	negative
Chicken	cloacal swabs tissues	1 23	negative negative
Cock	cloacal swabs tissues	2 1	negative negative
Cockatoo	cloacal swabs	5	negative
Flamingo	cloacal swabs tissues	25 3	negative negative
ostrich	cloacal swabs	34	negative
partridge	tissues	2	negative
pigeon	tissues	70	negative
psittacine birds	cloacal swabs tissues	237 13	negative negative
spanish imperial eagle	cloacal swabs	1	negative
tawny owl	cloacal swabs	1	negative
turkey	cloacal swabs	2	negative

Influenza viruses isolated

None.

SWEDEN

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broiler breeders	tissues cloacal swabs	22 55
pigeons	tissues	1
wild birds	tissues* cloacal swabs	98 15

* plus 9 tissue samples on cell cultures

Influenza viruses isolated

1 x H3N2 from wild bird.

Serological monitoring for avian influenza antibodies

Type of birds	No. flocks tested	No. sera examined	No. flocks positive	No. sera positive
breeders	93	5575	0	0
Imported breeders in quarantine	14	280	0	0
turkeys	6	300	0	0
wild birds		152	0	0

SWITZERLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
laying hens	tissues	1
broilers	tissues	29
turkeys	tissues	3
pigeons	tissues	1
pet birds	tissues	19

Influenza viruses isolated

None.

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

Type of poultry	Number of flocks tested	Number of sera examined	Number of flocks positive	Number of sera positive
laying hens	31	442	3	11

UK - GREAT BRITAIN

Samples tested

Type of bird	Sample	Method	Number
Chickens	tissues	eggs	244
	tissues	cell cultures	226
	cloacal swabs	eggs	16
	cloacal swabs	cell cultures	14
Turkeys	tissues	eggs	45
	tissues	cell cultures	40
Game birds	tissues	eggs	134
	tissues	cell cultures	34
	cloacal swabs	eggs	3
	cloacal swabs	cell cultures	3
Pigeons	tissues	eggs	145
	tissues	cell cultures	207
	cloacal swabs	eggs	28
	cloacal swabs	cell cultures	28
Waterfowl	tissues	eggs	54
	tissues	cell cultures	54
	cloacal swabs	eggs	88
Caged birds	tissues	eggs	163
	tissues	cell cultures	174
	cloacal swabs	eggs	3
	cloacal swabs	cell cultures	4
Raptors	tissues	eggs	7
	tissues	cell cultures	57
	cloacal swabs	eggs	1
	cloacal swabs	cell cultures	1

Influenza viruses isolated

Bird	subtype	IVPI	conclusion
Broiler breeders [one flock 7 isolates]	H10N7	0.00	LPAI
European white-fronted goose [wild]	H11N9	0.00	LPAI

UK - NORTHERN IRELAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Chickens	tissues cloacal swabs	21 15
Pigeons	tissues	37
Pheasants	tissues	8

Influenza viruses isolated

None

Influenza serology

Type of bird	Number Tested			
	HI H5N3	HI H7N7	HI H1N1 sw/Belg/79	HI H1N1 sw/Eng/92
chicken	55	55	0	0
turkeys	83	83	19	19
exotic	34	34	0	0

All with negative results

DISCUSSION

Of the 29 laboratories sent questionnaires a total of 24 laboratories responded, 14 EU laboratories and 10 non-EU. This represents an increase of one EU and 3 non-EU laboratories over the returns for 2000 (Alexander & Manvell, 2002).

The amount of diagnostic and surveillance work for avian influenza varied enormously with country from none to considerable diagnosis in the face of outbreaks of LPAI infections in poultry and wide scale surveillance of domestic and wild birds. The overall isolation attempts for avian influenza are summarised in Table 1. Interestingly almost three fold more tissue samples were examined than cloacal swabs or faeces despite the general consensus that the latter samples are likely to contain more virus and are easier to collect. This is probably a product of the passive nature of the sampling, depending primarily on diagnostic submissions.

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

Type of bird	Number countries reporting attempts	Number samples T = tissues C = cloacal swabs/faeces	Total samples
chickens	18	T 2111 C 490	2601
turkeys	9	T 475 C 152	627
ducks & geese	8	T 148 C 263	411
game birds	6	T 788 C 10	798
backyard/other poultry	5	T 39 C 16	55
ostriches	5	T 60 C 2	62
pigeons	15	T 954 C 68	1022
cage, zoo etc	12	T 1232 C 69	1301
wild birds	11	T 491 C 1130	1621
	TOTALS	**T 6298** **C 2200**	**8498**

A total of 47 influenza viruses was isolated from the 8498 attempts and was obtained from only six of the 24 countries (Table 2). However, over half [24] of the viruses isolated were associated with the continuing presence of H7N1 LPAI virus in Italy following the LPAI and HPAI epidemics in 1999-2000. In addition, 7 viruses were

obtained from a single broiler breeder flock infected with LPAI of H10N7 subtype in Great Britain and 7 H7N7 viruses were obtained from a single mixed species flock in Germany.

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

Country	Type of bird	Number	Subtype
France	meat turkeys	2	H6N2
	sentinel ducks	2	H6N2
Germany	turkeys[1]	1[1]	H7N7
	chickens	6[1]	H7N7
	pigeon	1	?
Italy	layers	1	H7N1
	meat turkeys	23	H7N1
Portugal	quarantine caged birds	2	H3N8
Sweden	wild bird	1	H3N2
UK Great Britain	broiler breeders[2]	7[2]	H10N7
	wild goose	1	H11N9

[1]single flock mixed birds [2]single flock

Serological testing for influenza varied enormously with laboratory ranging from planned active surveillance in Germany, through testing only imported birds, to no reported testing in 10 of the countries returning a questionnaire.

Only four countries reported detecting birds with antibodies to influenza A viruses as a result of surveillance. In Bulgaria use of an ELISA test for type A influenza viruses detected positive results [12.1 % overall] in ducks, pheasant and partridge sera. While the Swiss laboratory recorded 11/410 [2.5%] positive sera from hens using a similar ELISA test. In both France and Germany sera HI positive to H6 were detected following H6 virus isolations. As reported in the past a number of turkeys were shown to have antibodies to H1 antigen in Germany. The only report of antibodies to H5 or H7 antigens was in birds samples as a result of the infection of a mixed flock with virus of H7N7 subtype.

Overall the surveillance results suggest that the prevalence of influenza in European poultry and other birds is low and that there was no evidence of spread from the outbreaks in Italy to other countries. However, the proposed active surveillance to take place in EU countries during 2003 will represent a much more severe test of the true situation.

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IMMUNIZATION WITH PLASMID DNAS ENCODING HEMAGGLUTININ AND MATRIX GENES SUPPRESSES EXCRETION OF LOW PATHOGENIC H7 AVIAN INFLUENZA AFTER HOMOLOGOUS CHALLENGE

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Since there are evidences that low pathogenic (LP) avian influenza viruses (AIV) of the H5 and H7 subtypes can mutate into highly pathogenic (HP) AIV after circulation in poultry, there is a need to control H5 and H7 LP infections. In those situations, where there are high densities of poultry, contacts with wild birds or when turkeys are concerned, vaccination can afford a supplementary mean together with sanitary measures. There are few studies concerning set up and evaluation of vaccine against H7 AIV ; in addition only inactivated H7 AIV vaccines are available in case of emergency. Discrimination between H7 AIV vaccinated and H7 AIV infected poultry is tricky and requires serologic surveillance of unvaccinated sentinel birds. Even "DIVA strategy", based on the detection of NA antibodies specific to a vaccinal strain displaying an heterologous neuraminidase compared to the epizootic strain, is unable to detect reassortant viruses exhibiting a new NA subtype. Futhermore efficacy of inactivated vaccines is mainly measured towards clinical protection after lethal challenge with an HPAIV. But this criterion is inadequate because vaccinated poultry that do not display any sign of the disease after infection can excrete the virus, being a contamination source for other sensitive birds. This is the reason why we attempt to develop an H7 AIV vaccine including the sole viral proteins that are essential for complete protection against a broad spectrum of viral strains more or less phylogenetically related. Plasmid DNAs (pCMVHA, pCMVNP and pCMVM) respectively encoding avian influenza hemagglutinin, nucleoprotein and matrix protein from the low pathogenic H7N1 strain A/Chicken/Italy/1067/99 were constructed and administered to 5 weeks old specific pathogen free chickens. pCMVHA was used to determine the best injection method and the optimal quantity to inject according to hemagglutination inhibition (HI) response. The 100µg dose of pCMVHA administered with the Medijector, a needle free device, gave the best results. Subsequently administration of pCMVHA, pCMVHA+pCMVM and pCMVHA+pCMVNP were evaluated based on HI response and viral isolation after homologous challenge. Our results show that coadministration of pCMVHA and pCMVM at doses ranging from 50 to 100µg of each plasmid DNA, suppressed excretion in both tracheal and cloacal route in 25/25 chickens, that was not the case for other vaccine preparation.

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COMPARATIVE STUDIES OF THE PATHOGENICITY OF AVIAN INFLUENZA VIRUS ISOLATES FOR CHICKENS AND TURKEYS

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Summary

Nine influenza virus isolates of various subtypes were tested for their pathogenicity by determination of the intravenous pathogenicity index (IVPI) in chickens and turkeys. For low pathogenic avian influenza viruses the IVPI determined in chickens was in most cases 0, but did not always reflect the severity of the disease in the flock of origin. Turkeys were more susceptible for low pathogenic avian influenza viruses and showed higher IVPI's than chickens, although the difference was usually small. However two of the low pathogenic H7 viruses investigated showed a major difference in IVPI between chickens and turkeys independent of the species of origin. But also in these cases the IVPI in turkeys did not reach the limit of the high pathogenicity. Highly pathogenic avian influenza viruses had nearly the same IVPI in chickens as well as in turkeys. The pathological findings lead to the conclusion, that turkeys are more susceptible to influenza virus induced pancreas and kidney alterations than chickens.

Introduction

The clinical severity of avian influenza in domestic poultry ranges from asymptomatic infection, mild respiratory disease to severe systemic disease with 100% mortality. The main factor responsible for this variability is the extreme differences of the pathogenicity of the influenza viruses.

For the highly pathogenic form of avian influenza control measures are laid down by the European Economic Community in the Council Directive 92/40/EEC to be applied in the event of an outbreak in one of the member states (CEC, 1992). In this directive highly pathogenic avian influenza is defined as an infection of poultry caused by any influenza A virus which has an intravenous pathogenicity index (IVPI) in six-week-old chickens greater than 1.2 or any infection with influenza A virus of H5 or H7 subtype which have multiple basic amino acids at the cleavage site of the haemagglutinin.

Another factor influencing the severity of the disease caused by avian influenza virus is the species infected (Alexander et al., 1986). Chickens and turkeys are considered to be highly susceptible and they are the most widely and the most intensively farmed avian species. Therefore it is of particular importance to know the pathogenicity of an influenza virus for these species, regardless of the original host of the virus. Because chickens are generally available as specific pathogen free laboratory animals, this

species is used for the intravenous pathogenicity tests, as regulated by the Council Directive 92/40/EEC.

In Germany some infections of turkeys with different influenza viruses of low pathogenicity have been reported in the last years (Sieverding, 1997; Werner, 1999) but outbreaks in chickens have not been registered. All isolated viruses had IVPIs in six-week-old chickens of zero, although in some cases moderate to severe symptoms were seen in the turkey flocks of origin.
Therefore the question arises, whether the IVPI in such cases should rather be determined in turkeys.

Materials and Methods

To investigate the possibility that influenza viruses of turkey origin result in a higher IVPI in turkeys than in chickens, we tested some of the influenza viruses of various subtypes (H9N2, H6N1, H6N2, H6N5) isolated from turkeys in Germany (Table 1). For comparison of their pathogenicity they were studied in turkeys and chickens simultaneously. In addition we examined an H5 virus (Fioretti et al.,1998) and different H7 viruses that caused influenza outbreaks in Italy (Capua et al.,1999).

Molecular biological investigation of the cleavage site of the haemagglutinin revealed multiple basic amino acids only in the two H7N1 viruses isolated in Italy in December 1999 (Table 2).

All other viruses had an amino acid sequences typical for low pathogenic influenza virus.

For the intravenous pathogenicity tests we used 6-week-old White Leghorn chicks (VALO SPF, Lohmann Tierzucht, Cuxhaven) and 5-week-old female turkey poults Big 6 (Moorgut Kartzfehn). The birds were purchased at day one of age and reared in isolated floor pen units of the quarantine animal house of our institute.

They were free of antibodies to influenza virus at the time of starting the experiments.

The IVPI tests were done as described in the Council Directive 92/40/EEC.

The animal experiments were performed in isolated rooms for each virus in the high security animal house.Chickens and turkeys, which received the same virus, were housed in separate cages in the same room.

Results

The results are summarized in table 3.

All H6 and also the H9 and H5 virus isolates had an IVPI of 0 in chickens. The birds did not show any clinical signs. But in three tests some turkeys showed, for one or more

days, decreased activity, ruffled feathers and/or diarrhoea. In the test with H6 virus R26 some turkeys were sick at day 4 and 5. After this they recovered. In the test with H5 virus some turkeys were sick from day 4 to day 9, but all birds were normal at the end of the observation period.

At necropsy after killing at the end of the experiment turkeys as well as chickens had pathological changes, although clinical symptoms were not longer observed at this time. The most prominent and consistent findings were a moderately enlarged pancreas with multiple variably sized, greyish-white foci of necrosis. The kidneys were slightly swollen, brownish with grey partly confluent foci. In general the lesions were more severe in turkeys than in chickens. Slightly increased mucous secretion in larynx and trachea were observed only in chickens as well as sporadic small dark red foci in the mucosa of the intestine.

The table 4 scores the differences of the frequency and the severity of pathological alterations in different organs in chickens and turkeys.

Much more marked differences in the pathogenicity for chickens and turkeys were seen for the low pathogenic H7 isolates from Italy 1999. The strain 472 had an IVPI of 0 in chickens, but 0.87 in turkeys. None of the chickens showed any clinical signs whereas all turkeys showed at least moderate signs and one turkey died at day 7 after inoculation.

The strain 473 isolated from chickens had an IVPI of 0.19 in chickens, but 0.63 in turkeys. Some chickens showed slight transient depressions and diarrhoea. On the other hand a greater number of turkeys were sick with more severe diarrhoea then the chickens. One turkey had respiratory signs and died at day six (Table 5).

The pancreas of all diseased turkeys was markedly enlarged, diffusely greyish-white with focal white necrotic areas surrounded by haemorrhage. The kidneys were diffusely swollen, and showed grey to pale yellow partly confluent foci. Histopathologically there were severe diffuse lymphocytic and histiocytic, focally necrotizing pancreatitis and nephritis. In all cases the lesions were more severe in turkeys than in chickens.

The two turkeys that died had a congestion of all parenchymal organs, moderate pancreatitis, nephritis and enteritis. The most prominent lesions were a fibrinous laryngitis and partly obstructive tracheitis, which appeared to have caused the death by suffocation.

The H7N1 strains 444 and 445 isolated in Italy in December1999 had very high IVPIs in agreement with the molecular biological findings. There were no relevant differences between chickens and turkeys. In all groups most animals died within 24 h and all were dead at the second day after inoculation.

At necropsy there was severe congestion in liver, lungs and kidneys. The pancreas of most animals was enlarged and showed vitreous sharply demarcated pinhead-sized foci. The proventriculus was filled with large amounts of white mucous. In the intestine moderate diffuse enteritis was present characterized by dark red small areas in the

mucosa and a greenish mucoid content. The myocardium was sometimes spotted and streaky due to a severe multifocal myocarditis. Subepicardial haemorrhages were only occasionally present.

The lesions were nearly the same in chickens and turkeys.

Conclusions

- Highly pathogenic avian influenza viruses can be detected equally as well by IVPI tests performed in chickens or in turkeys.
- For low pathogenic avian influenza viruses the IVPI determined in chickens is in most cases 0, but does not always reflect the severity of the disease in the flock of origin.
- Turkeys are more susceptible for low pathogenic avian influenza viruses and show higher IVPI's than chickens, although the difference is usually small.
- However the Italian H7 low pathogenic viruses from 1999 that were investigated show a major difference in IVPI between chickens and turkeys independent of the species of origin. But also in these cases the IVPI in turkeys does not reach the limit of the high pathogenicity (IVPI above 1.2).
- The pathological findings lead to the conclusion, that turkeys are much more susceptible to influenza virus induced pancreas and kidney alterations than chickens. This can be stated at least for the genetic lines of birds used.

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Table 1. ***Influenza viruses comparatively tested for pathogenicity in chickens and turkeys***

Virus		Disease in the flock of origin
A/turkey/Germany/R05/96	(H9N2)	moderate
A/turkey/Germany/R43/98	(H6N5)	mild
A/turkey/Germany/R26/99	(H6N2)	moderate
A/turkey/Germany/R30/99	(H6N1)	mild
A/chicken/Italy/22/98	(H5N9)	mild
A/turkey/Italy/472/99	(H7N1)	moderate
A/chicken/Italy/473/99	(H7N1)	moderate
A/chicken/Italy/444/99	(H7N1)	severe
A/chicken/Italy/445/99	(H7N1)	severe

Table 2. Determination of pathogenicity by nucleotide sequencing

Subtyp	Virus	Cleavage site of the hemagglutinin	Pathogenicity
H9	tk/G/R05/96	NVPAA - - - - - - SAR∗GLFG	low
H6	tk/G/R43/98	NVPQI - - - - - - ETR∗GLFG	low
	tk/G/R26/99	NVPQI - - - - - - ETR∗GLFG	low
	tk/G/R30/99	NVPQI - - - - - - ETR∗GLFG	low
H5	ch/It/22/98	NVPQ - K - - - - - ETR∗GLFG	low
H7	tk/It/472/99	NVPEIP KG - - - - R∗GLFG	low
	ch/It/47399	NVPEIP KG - - - - R∗GLFG	low
	ch/It/444/99	NVPEIP KGSRVRR∗GLFG	high
	ch/It/445/99	NVPEIP KGSRVRR∗GLFG	high

Table 3. ***Results of intravenous pathogenicity tests in chickens and turkeys***

Virus	*Subtype*	*IVPI*	
		chickens	*turkeys*
tk/G/R05/96	***H9***	***0***	***0***
tk/G/R43/98	***H6***	***0***	***0.02***
tk/G/R26/99	***H6***	***0***	***0.13***
tk/G/R30/99	***H6***	***0***	***0***
ch/It/22/98	***H5***	***0***	***0.18***
tk/It/472/99	***H7***	***0***	***0.87***
ch/It/473/99	***H7***	***0.19***	***0.63***
ch/It/444/99	***H7***	***2.94***	***2.99***
ch/It/445/99	***H7***	***2.98***	***3.00***

Table 4. Differences in gross lesions in chickens and turkeys after intravenous infection with low pathogenic avian influenza viruses

Species	**post-mortem findings**			
	laryngitis/tracheitis	**enteritis**	**pancreatitis**	**nephrosis/nephritis**
Chickens	++[b]	++	+	+
Turkeys	+[a]	+	+++[c]	+++

[a] uncommon (seldom), mild form
[b] common, moderate lesions
[c] common, severe alteration

Table 5. ***Intravenous Pathogenicity Index (IVPI) of A/chicken/Italy/473/99 (H7N1)***

Chickens:

Clinical Signs	Day after inoculation										Total Score
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Normal	10	10	10	10	8	6	4	5	8	10	81 x 0 = 0
Sick	0	0	0	0	2	4	6	5	2	0	19 x 1 = 19
Severely sick (*)	0	0	0	0	0	0	0	0	0	0	0 x 2 = 0
Dead	0	0	0	0	0	0	0	0	0	0	0 x 3 = 0
											Total = 19

Index = mean score per bird per observation $= \frac{19}{100} =$ 0,19

Turkeys:

Clinical Signs	Day after inoculation										Total Score
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Normal	10	10	10	3	2	3	3	3	2	2	48 x 0 = 0
Sick	0	0	0	7	7	6	6	6	7	7	46 x 1 = 46
Severely sick (*)	0	0	0	0	1	0	0	0	0	0	1 x 2 = 2
Dead	0	0	0	0	0	1	1	1	1	1	5 x 3 = 15
											Total = 63

Index = mean score per bird per observation $= \frac{63}{100} =$ 0.63

STRATEGIES FOR THE CONTROL OF AVIAN INFLUENZA IN ITALY

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SUMMARY

The present paper describes the control measures applied during the 1999-2001 Italian avian influenza epidemic. The epidemic was caused by a low pathogenicity avian influenza virus of the H7N1 subtype that mutated into a highly pathogenic avian influenza virus, after circulating in the industrial poultry population for approximately nine months. Following the emergence of the HPAI virus, that caused death or culling of over 13 000 000 birds, and the implementation of the measures indicated in Directive 92/40/CE, the LPAI virus re-emerged twice.

In order to control the re-emergence of LPAI virus and to develop a novel control strategy, a "DIVA" (Differentiating Vaccinated from Infected Animals) strategy was developed and combined to a strict territorial control programme. The "DIVA" strategy was based on the use of an inactivated oil emulsion vaccine containing the same haemagglutinin (H) subtype as the field virus, but a different neuraminidase (N). The possibility of using the diverse N group, to differentiate between vaccinated and naturally infected birds, was achieved through the development of an "*ad hoc*" serological test based on the detection of specific anti- N1 antibodies.

The control of the field situation was ensured through an intensive sero-surveillance programme aiming at detecting the circulation of the LPAI virus, through the regular testing of vaccinated and unvaccinated flocks, the latter located both inside and outside the vaccination area. In addition, the efficacy of the vaccination schemes was evaluated in the field through regular testing of selected flocks.

After the first year of vaccination, the epidemiological data collected, indicating that the H7N1 virus was not circulating in the vaccinated poultry, was considered to be sufficient by the EU Commission to lift the marketing restrictions on meat obtained from vaccinated poultry.

The experience gained during the Italian 1999-2001 AI epidemic, suggest that the combination of a "DIVA" control strategy with a territorial monitoring system under official control can represent an effective tool for the control of avian influenza infections in poultry. In addition, the application of a "DIVA" vaccination policy, as opposed to a conventional policy enabled veterinary public health organisations to establish that infection was not circulating any longer, and ultimately resulted in the possibility of marketing meat obtained from animals vaccinated against an OIE List A disease.

INTRODUCTION

Highly pathogenic avian influenza (HPAI) is a viral disease of poultry included in OIE List A, and in the European Union its control is imposed by EU Directive 92/40/EEC (4). The disease may have devastating effects on the poultry industry particularly when it affects industrial poultry rearing systems, and its presence in a given territory results in restrictions on animal movements, marketing and trade of poultry and poultry products. Similar restrictions are enforced if vaccination for HPAI is implemented in a given territory.

THE ITALIAN 1999-2001 H7N1 EPIDEMIC

During 1999 and 2000 north-eastern Italy has been affected by a devastating epidemic of HPAI, caused by a type A influenza virus of the H7N1 subtype that originated from the mutation of a low pathogenicity avian influenza (LPAI) virus of the same subtype (1). The HPAI epidemic caused directly or indirectly the death or culling of over 13 million birds that inevitably determined the disruption of the marketing system and great economic losses to the poultry industry and to the social community. Following depopulation and restocking of the HPAI infected areas, LPAI re-emerged twice, thus determining the poultry industry to request, through the Italian veterinary authorities, and obtain vaccination against avian influenza of the H7 subtype.

VACCINATION POLICY

Following the second LPAI epidemic wave, a vaccination policy against avian influenza was, strongly requested by the farmers and by the poultry industry. However, document Sanco/B3/AH/R17/2000 of the EU Scientific committee on animal health and animal welfare, stated that a vaccination programme could be approved provided that the vaccine administered was a non genetically modified vaccine, and that it could be possible to discriminate vaccinated from infected birds.

The strategy proposed by the Italian reference laboratory was that of using an inactivated oil emulsion vaccine containing a strain with a homologous haemagglutinin (H) group and a heterologous neuraminidase (N) group. The reason for this is the possibility of using it as a natural "marker" vaccine, or more correctly a DIVA [Differentiating Infected from Vaccinated Animals] vaccine. In fact it is well known the neutralizing antibodies to influenza A viruses are induced primarily by the haemagglutinin molecule (4) and that vaccination with a vaccine containing a homologous H group also reduces the amount of infectious virus shed in the environment (4). Therefore the use of a vaccine containing the H7 antigen would ensure protection against clinical signs and, in case of infection the reduction of virus shedding. The presence of a different neuraminidase (N) subtype, which induces the production of specific antibodies, would enable official veterinary authorities, with the aid of an *"ad hoc"* diagnostic test, to discriminate between infected and vaccinated flocks, and to monitor and follow the evolution of the situation.

Following the satisfactory results of cross-protection studies against the Italian H7N1 virus, (Capua, unpublished) the vaccine that has been used in the field has been

prepared from an inactivated H7N3 virus (A/CK/Pakistan/95/H7N3). The discrimination between infected (N1 positive) and vaccinated (N1 negative) birds was achieved by an indirect immunofluorescence test (iIFAT), based on a recombinant N1 protein expressed in a baculovirus vector (3).

A control strategy based on the combination of a complex field monitoring system, and on the application of strict vaccination protocols complying to those evaluated in the laboratory and on the implementation of the “DIVA” strategy was drawn up and approved by the, EU commission (2000/721/EC).

The vaccination program began on November 15th 2000 and will last until May 2002. Approximately 12 000 000 birds [only meat type birds and table-egg layers (that apply the all-in all-out system)] raised in a restricted zone (1156 km^2) south of Verona will be involved in the vaccination programme. The vaccine is being marketed only under strict official control, and until November 30th, 2001 no vaccinated live birds or poultry products that originated from the vaccination zones were authorised for intra-community trade.

TERRITORIAL STRATEGY

The territorial strategy was drawn in order to constantly monitor the situation in the field through official controls and voluntary notification. Briefly, the identification of any LPAI infected farms relied on the notification of suspected cases by farmers and field veterinarians. In addition, official veterinarians carried out regular serological testing of poultry flocks at risk of infection. Once an infected flock was identified, an epidemiological investigation was carried out in the affected farms by means of a standardized questionnaire to establish the possible origin of the infection and to identify flocks directly or indirectly at risk of infection. In case of the identification of an outbreak, although no compulsory eradication or compensation for LPAI is provided for in EU legislation, the Italian Ministry of Health opted for eradication. This was achieved by stamping out or by controlled marketing of slaughterbirds, on infected farms. In addition, the prohibition of restocking of poultry farms, and the enforcement of restriction measures on the movement of live poultry, vehicles and staff were imposed in the areas at risk.

Official monitoring of the areas at risk was implemented with the aim of identifying undiagnosed outbreaks and to evaluate the efficacy of the vaccination program in the field.

The control of the vaccinated farms was achieved both through regular testing of sentinel birds and through the application of the N1-N3 discriminatory test. In fact, each vaccinated flock contained at least 1% of unvaccinated birds, which were appropriately identified to distinguish them from vaccinated animals. In vaccinated flocks at least 10 sentinel birds (95% probability to identify at least one positive bird if the prevalence of seropositive animals is ≥ 30%) were serologically tested every 45 days. Alternatively 10 serum samples were collected from each shed and processed for the N1-N3 discriminatory test.

In addition, a monitoring program to evaluate the efficacy of the vaccination protocols in the field was also carried out, with the regular serological testing of 20 vaccinated animals in at least 30 randomly selected vaccinated farms (95% probability of identifying at least one non-immunized flock if their frequency is ≥ 10%).

In order to adhere to the vaccination campaign, farmers had to comply with strict biosecurity measures, and undergo regular inspections under official control.

RESULTS

Following the implementation of the vaccination policy, the third LPAI epidemic wave was detected in a non-vaccinated meat-type turkey farm located in the vaccination area. A total of 23 outbreaks (22 meat-type turkey and 1 layer farms) were identified and the last affected flock was stamped out on the 26th of March 2001. During this epidemic wave, only 3 meat-type turkey farms were infected inside the vaccination area and among these, only one vaccinated flock was affected. The presence of infection was promptly identified through the laboratory investigations performed on the sentinel birds. The farm was located in close proximity (200 m) to an unvaccinated farm and the infection did not spread further to neighboring (0.8 - 1 km) vaccinated farms

The intensive sero-surveillance programme, applied to poultry farms located outside the vaccination area in the Veneto region, following the eradication of the last LPAI outbreak, determined the sampling and processing of over 35.000 blood samples with negative results.

The N1-N3 discriminatory test was also used to assess whether serological positivity to the HI test detected in sentinel birds was truly determined by infection or was due to sampling of vaccinated birds instead of sentinels. All samples were tested with negative results.

The data obtained from the intensive monitoring program were considered sufficient by the EU Member States, to demonstrate that the LPAI virus was not circulating any longer in the naïve or in the vaccinated poultry population. The application with negative results, of the N1-N3 discriminatory test, was considered as an additional guarantee for Member States, and on 30th November 2001, commission decision 2001/847/CE, authorized the marketing of meat obtained from vaccinated birds for intra-community trade.

DISCUSSION

With reference to control of avian influenza infections, the data presented herein indicate that the strategy implemented in Italy, i.e., the association between a territorial strategy under official control, strict biosecurity measures including restriction on animal movements and a “DIVA” vaccination program can be successful in controlling LPAI. In addition, the emergence of a HPAI strain, was avoided.

The comparison between the rates of spread observed during the 1999-2000 H7N1 LPAI epidemics, which occurred prior to the enforcement of this control strategy, and

the limited LPAI spread observed in the vaccinated area supports the association between restriction, bio-security, surveillance and vaccination as effective tools for the control of LPAI.

Critical points for the application of this strategy appear to be, firstly the development and validation of a tailored discriminatory test (in this case N1- N3) that enables the differentiation between infected and vaccinated birds, in a relatively short period of time. Secondly, the great amount of work on public officials that is required in order to develop a complex territorial approach, market and distribute the vaccine, collect blood samples for monitoring programs, inspect farms on a regular basis, perform laboratory tests and collect and analyze data must be carefully organized and planned for.

In addition, prompt diagnosis and the implementation of a stamping out or a controlled marketing policy on affected farms are fundamental to the eradication of LPAI infections.

In our opinion it is imperative that the results obtained from the territorial control strategy are 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.

ACKNOWLEDGEMENTS

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INFLUENZA SURVEILLANCE IN WILD BIRDS IN NORTHERN EUROPE

Ron Fouchier

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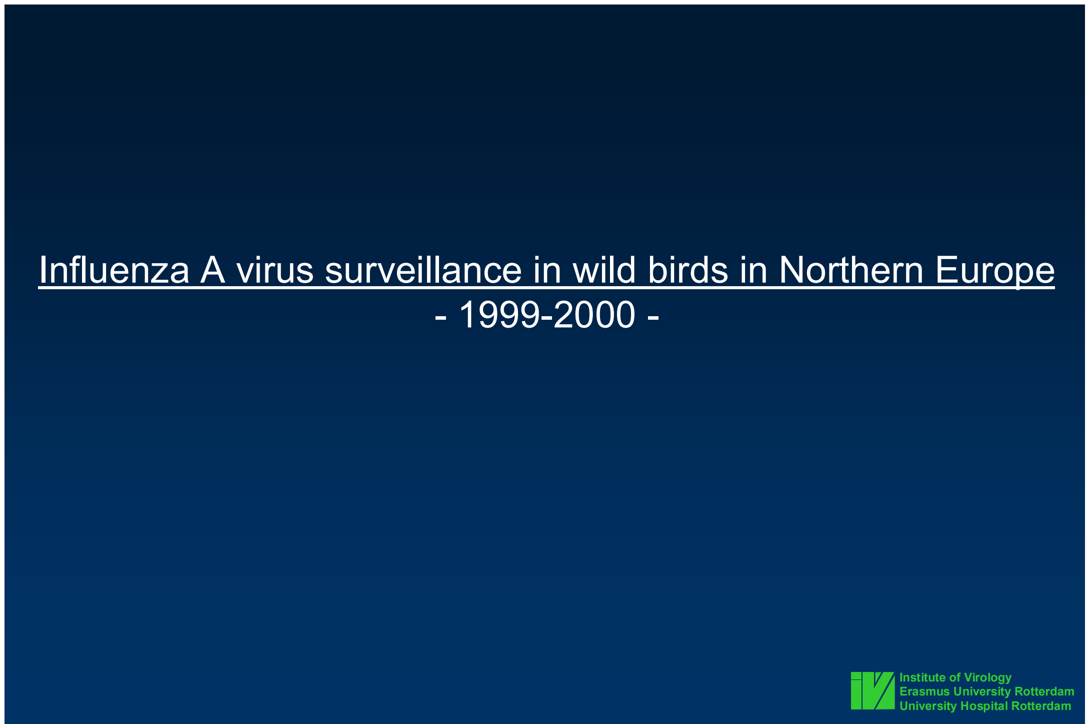

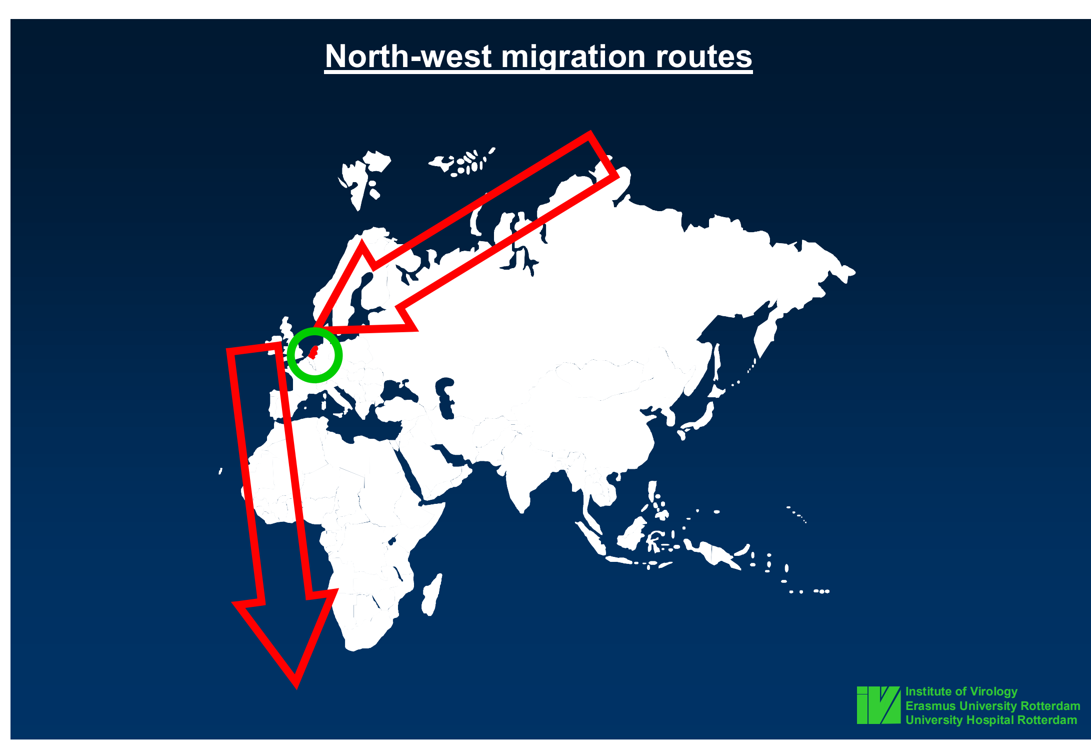
North-west migration routes
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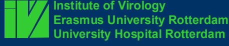
Erasmus University Rotterdam
University Hospital Rotterdam

Bird sample collection

The Netherlands	
Bird ring station Schiermonnikoog	Andre van Loon et al.
Bird ring station Castricum	Guido Keijl et al.
Bird ring station AWD Amsterdam	Hans Vader et al.
Bird ring station Mr. Cornelis van Lennep	Erik Maassen et al.
Ducks Eendenkooi Bakkerswaal Lekkerkerk	Hans Zantinge
Ducks Eendenkooi Krimpen a.d. Lek	Bert Pellegrom
Geese Groningen	John de Boer
Geese Vlieland	Dirk Kuiken
Geese Ameland	Jan Smit
Geese Terschelling	Harry Horn
Geese Gelderland	Dick Jonkers et al.
Gulls IJmuiden	Kees Verbeek, Fred Cottaar
Gulls Groningen	Corine Eising, Ton Groothuis, RUG
Plovers Oosterbierum	Joop Jukema
Birds of prey Friesland	Vogelasiel de Fûgelhelling, Ureterp

International	
Bird Observatory Ottenby, Sweden & birds Antarctica	Bjorn Olsen et al.
Fulmars Faroer Islands	Bjorn Herrman
Birds Iceland	Carla Dove
Birds Ghana, Nigeria	Ulf Ottosson, Jonas Waldenström
Birds Mauritania	C.N.R.O.P.
Poultry Bangkok	Parntep Ratanakorn



Bird-samples for influenza A virus surveillance; 1999 & 2000

Order	Family		# Species	# Samples
Procellariiformes	*Procellariidae*	Fulmar	1	101
Anseriformes	*Anatidae*	Goose	6	1387
		Duck	12	2232
		Swan	2	73
Falconiformes	*Accipitridae*	Hawk, Eagle, Buzzard	3	26
Gruiformes	*Rallidae*	Rail, Coot	4	281
Charadriiformes	*Charadriidae*	Plover, Lapwing	5	166
	Scolopacidae	Sandpiper, Dunlin, Snipe	23	1977
	Laridae	Gull	5	886
	Alcidae	Auk	2	28
	Burhinidae	Oystercatcher	1	31
Passeriformes	*Alaudidae*	Lark	1	61
	Prunellidae	Accentor	2	67
	Muscicapidae	Thrush, Warbler, etc.	26	740
	Paridae	Tit	4	99
	Sturnidae	Starling	1	52
	Fringillidae	Finch	5	99
	Motacillidae	Wagtail, Pipit	3	40
Others	Others	Others	17	35
		Poultry		406
			123 +	8787 +

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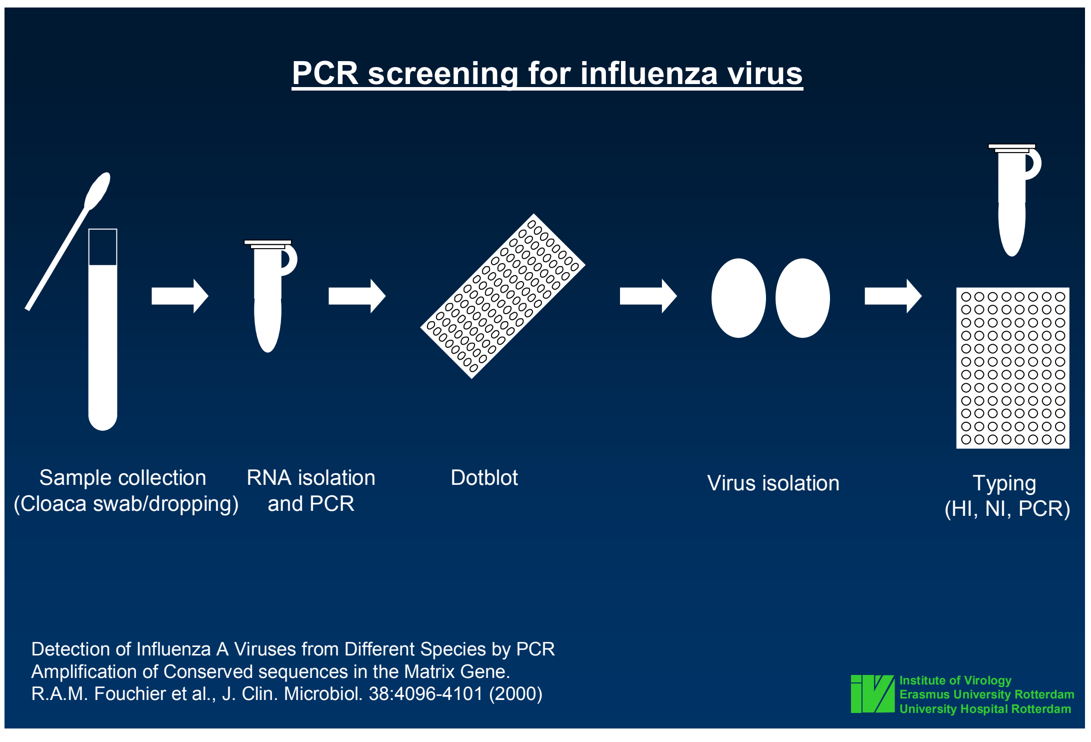

Results 1999 & 2000

	Species	N Tested	N PCR+	(%)	N Egg +
Ducks	Mallard	1180	39	(3.3)	22
	Teal	489	13	(2.7)	3
	Wigeon	399	4	(1.0)	2
	Shoveler	44	1	(2.3)	1
	Shelduck, Eider, Gadwall, Pochard,Pintail, etc.	120	0		0
Geese	White-fronted	504	13	(2.6)	5
	Greylag	271	7	(3.7)	4
	Brent, Barnacle, Bean, Egyptian	612	0		0
Gulls	Black-headed	453	10	(2.2)	6
	Common, Herring, Black-backed, Kittiwake	433	0		0
Others	Guillemot	27	3	(11.1)	1
Others	Other birds	4255	0		0
	+	8787	90	(1.0)	44

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Influenza A virus isolates from European birds, 1999 & 2000

- HA & NA subtypes -

HA	Species
1	Mallard, Teal
2	Mallard, White-fronted Goose
3	Mallard, Teal
4	Mallard
5	Mallard
6	Mallard, Wigeon, Greylag Goose, White-fronted Goose, Guillemot
7	Mallard
8	---
9	---
10	Mallard
11	Mallard, Teal, Shoveler
12	---
13	Black-headed Gull
14	---
15	---
XX	Black-headed Gull

NA	Species
1	Mallard, Teal
2	Mallard, Wigeon, Greylag Goose, White-fronted Goose, Guillemot
3	Black-headed Gull
4	Mallard, Wigeon
5	Mallard
6	Mallard, Black-headed Gull
7	Shoveler
8	Mallard, Teal, White-fronted Goose
9	---

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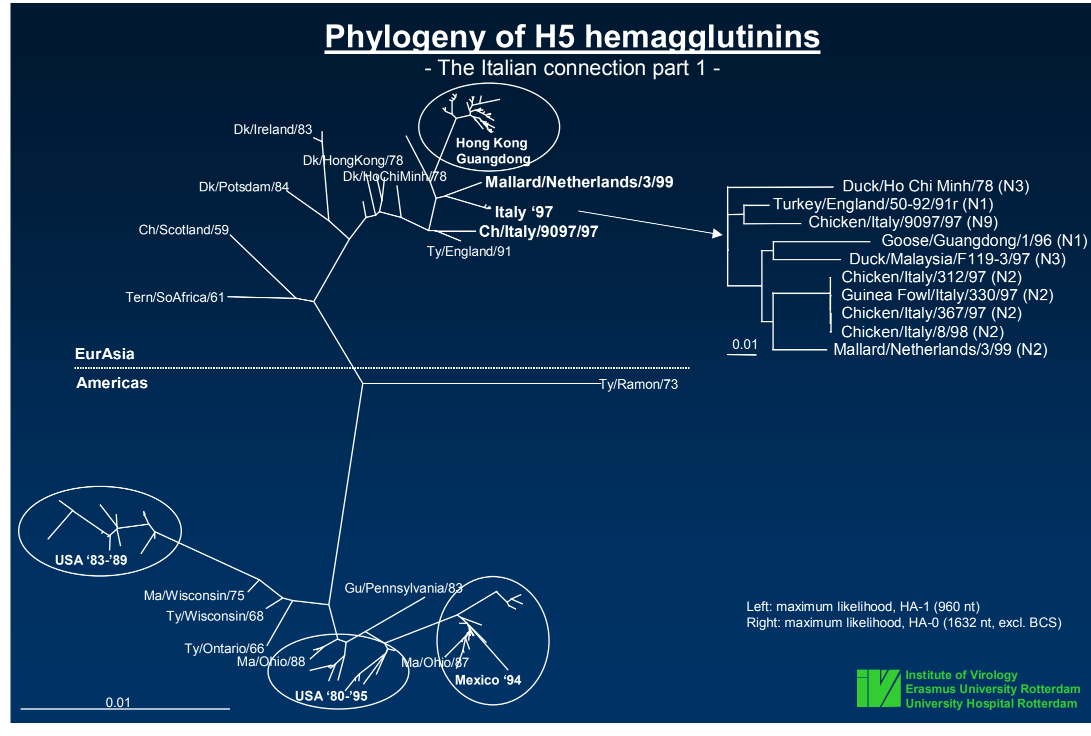

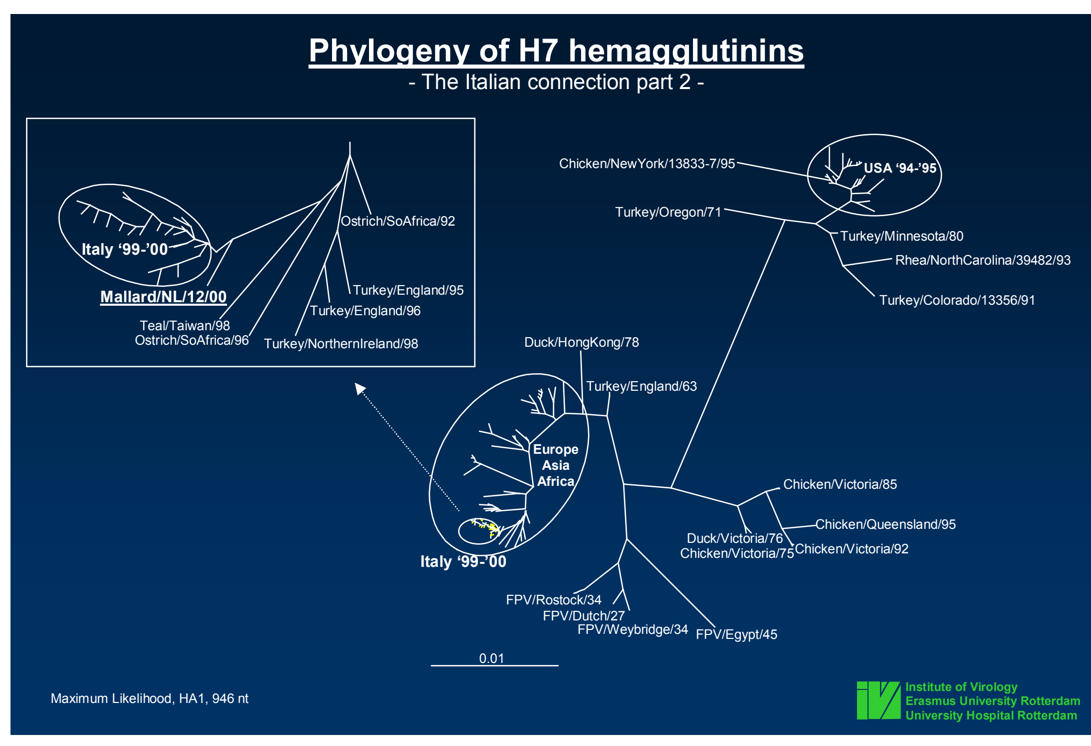

Phylogeny of H7 hemagglutinins
- The Italian connection part 2 -
Italy '99-'00
Mallard/NL/12/00
Ostrich/SoAfrica/92
Turkey/England/95
Turkey/England/96
Teal/Taiwan/98
Ostrich/SoAfrica/96
Turkey/NorthernIreland/98
Chicken/NewYork/13833-7/95
USA '94-'95
Turkey/Oregon/71
Turkey/Minnesota/80
Rhea/NorthCarolina/39482/93
Turkey/Colorado/13356/91
Duck/HongKong/78
Turkey/England/63
Europe
Asia
Africa
Italy '99-'00
Chicken/Victoria/85
Chicken/Queensland/95
Duck/Victoria/76
Chicken/Victoria/75
Chicken/Victoria/92
FPV/Rostock/34
FPV/Dutch/27
FPV/Weybridge/34
FPV/Egypt/45
0.01
Maximum Likelihood, HA1, 946 nt
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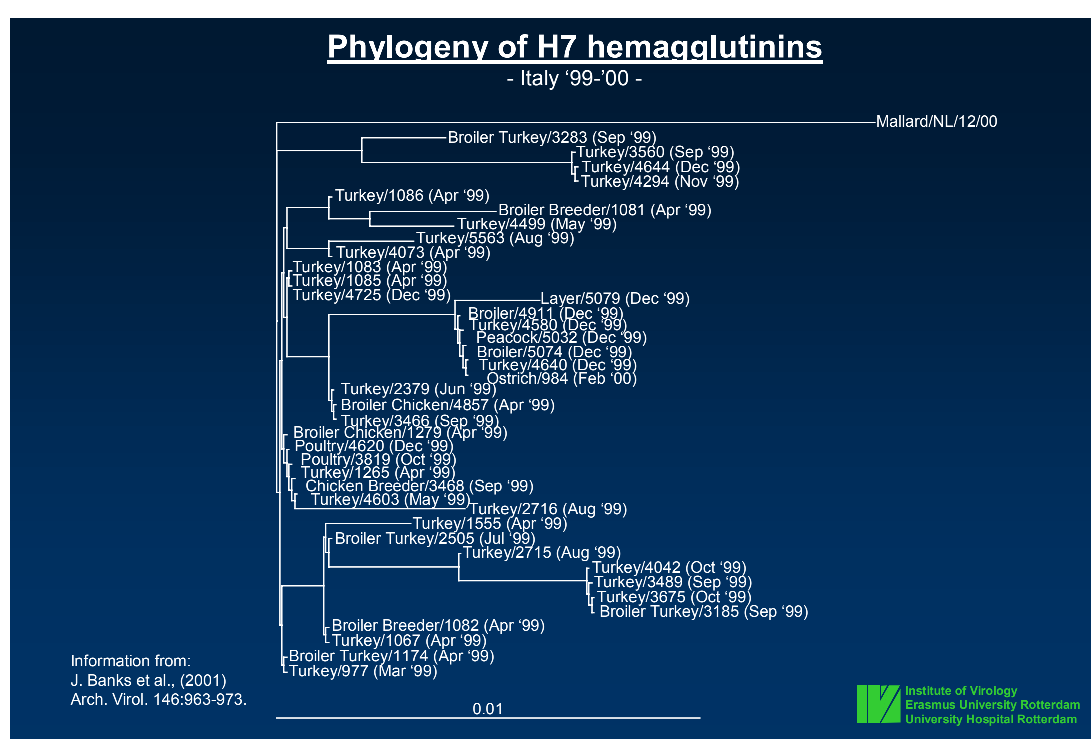

Phylogeny of H7 hemagglutinins
- Italy '99-'00 -
Mallard/NL/12/00
Broiler Turkey/3283 (Sep '99)
Turkey/3560 (Sep '99)
Turkey/4644 (Dec '99)
Turkey/4294 (Nov '99)
Turkey/1086 (Apr '99)
Broiler Breeder/1081 (Apr '99)
Turkey/4499 (May '99)
Turkey/5563 (Aug '99)
Turkey/4073 (Apr '99)
Turkey/1083 (Apr '99)
Turkey/1085 (Apr '99)
Turkey/4725 (Dec '99)
Layer/5079 (Dec '99)
Broiler/4911 (Dec '99)
Turkey/4580 (Dec '99)
Peacock/5032 (Dec '99)
Broiler/5074 (Dec '99)
Turkey/4640 (Dec '99)
Ostrich/984 (Feb '00)
Turkey/2379 (Jun '99)
Broiler Chicken/4857 (Apr '99)
Turkey/3466 (Sep '99)
Broiler Chicken/1279 (Apr '99)
Poultry/4620 (Dec '99)
Poultry/3819 (Oct '99)
Turkey/1265 (Apr '99)
Chicken Breeder/3468 (Sep '99)
Turkey/4603 (May '99)
Turkey/2716 (Aug '99)
Turkey/1555 (Apr '99)
Broiler Turkey/2505 (Jul '99)
Turkey/2715 (Aug '99)
Turkey/4042 (Oct '99)
Turkey/3489 (Sep '99)
Turkey/3675 (Oct '99)
Broiler Turkey/3185 (Sep '99)
Broiler Breeder/1082 (Apr '99)
Turkey/1067 (Apr '99)
Broiler Turkey/1174 (Apr '99)
Turkey/977 (Mar '99)
0.01
Information from:
J. Banks et al., (2001)
Arch. Virol. 146:963-973.
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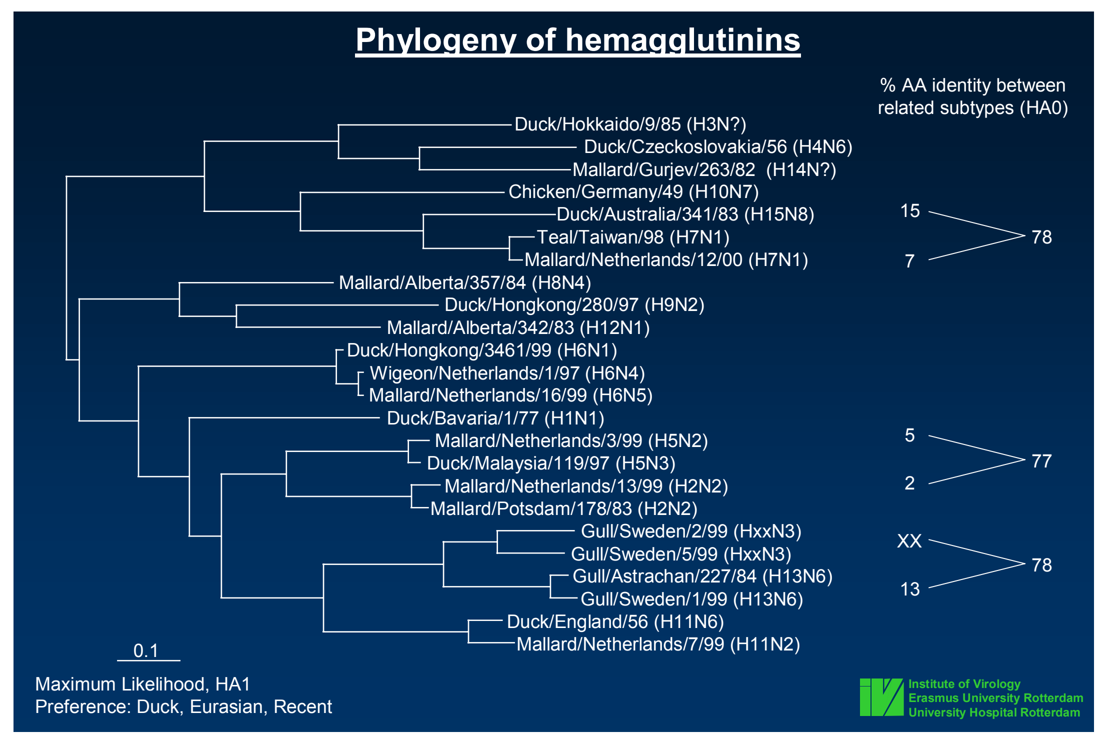
Phylogeny of hemagglutinins
% AA identity between related subtypes (HA0)
Duck/Hokkaido/9/85 (H3N?)
Duck/Czeckoslovakia/56 (H4N6)
Mallard/Gurjev/263/82 (H14N?)
Chicken/Germany/49 (H10N7)
Duck/Australia/341/83 (H15N8)
Teal/Taiwan/98 (H7N1)
Mallard/Netherlands/12/00 (H7N1)
Mallard/Alberta/357/84 (H8N4)
Duck/Hongkong/280/97 (H9N2)
Mallard/Alberta/342/83 (H12N1)
Duck/Hongkong/3461/99 (H6N1)
Wigeon/Netherlands/1/97 (H6N4)
Mallard/Netherlands/16/99 (H6N5)
Duck/Bavaria/1/77 (H1N1)
Mallard/Netherlands/3/99 (H5N2)
Duck/Malaysia/119/97 (H5N3)
Mallard/Netherlands/13/99 (H2N2)
Mallard/Potsdam/178/83 (H2N2)
Gull/Sweden/2/99 (HxxN3)
Gull/Sweden/5/99 (HxxN3)
Gull/Astrachan/227/84 (H13N6)
Gull/Sweden/1/99 (H13N6)
Duck/England/56 (H11N6)
Mallard/Netherlands/7/99 (H11N2)
15
7
78
5
2
77
XX
13
78
0.1
Maximum Likelihood, HA1
Preference: Duck, Eurasian, Recent
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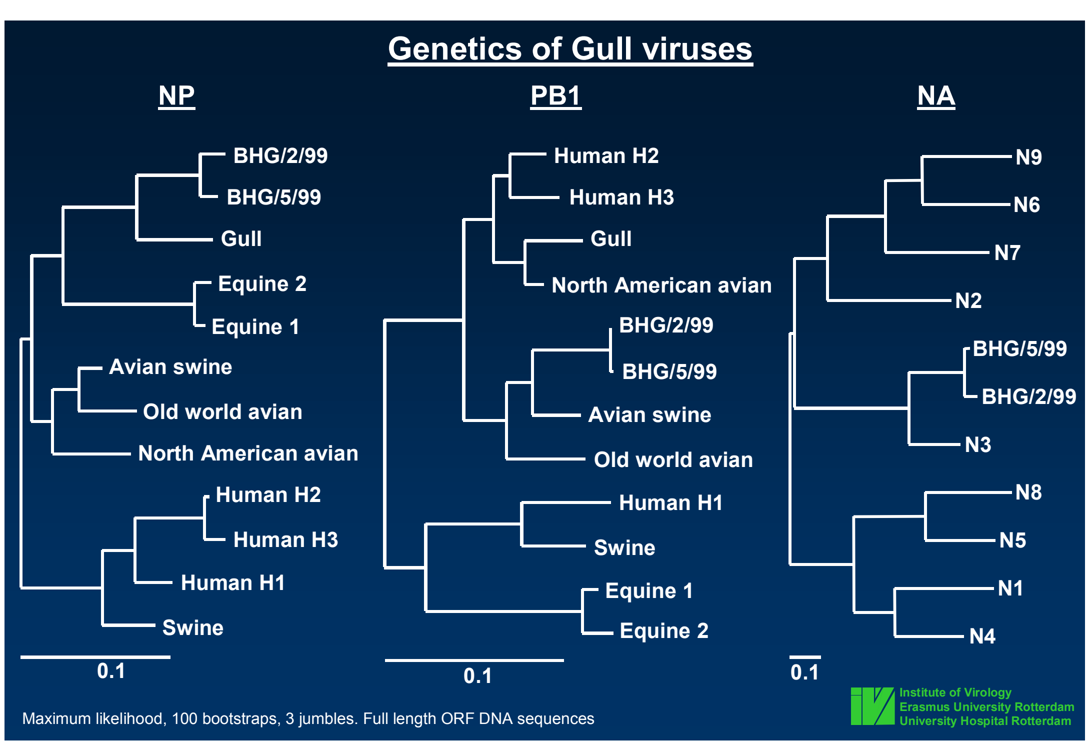
Genetics of Gull viruses
NP
BHG/2/99
BHG/5/99
Gull
Equine 2
Equine 1
Avian swine
Old world avian
North American avian
Human H2
Human H3
Human H1
Swine
0.1
PB1
Human H2
Human H3
Gull
North American avian
BHG/2/99
BHG/5/99
Avian swine
Old world avian
Human H1
Swine
Equine 1
Equine 2
0.1
NA
N9
N6
N7
N2
BHG/5/99
BHG/2/99
N3
N8
N5
N1
N4
0.1
Maximum likelihood, 100 bootstraps, 3 jumbles. Full length ORF DNA sequences
Institute of Virology
Erasmus University Rotterdam
University Hospital Rotterdam

Influenza A virus surveillance in wild birds in Northern Europe - Conclusions -

PCR-based screening

A rapid, sensitive alternative to classical screening for influenza A virus

Surveillance

1-3 % of some duck, goose, gull species positive for influenza A virus late fall, early winter
No influenza A virus in many species of birds, including certain duck, goose species, shorebirds
Many HA and NA subtypes in a small geographical area, even individual marshalling lakes
Close relatives of Italian HPAI in wild ducks in The Netherlands

Gull influenza

Novel HA subtype?
The result of reassortant (Eurasian PB1, NA) genomes?

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Monique Spronken		Nella Nieuwkoop	
Emmie de Wit		Theo Voeten	
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Kalmar County Hospital
Birdobservatory Ottenby

Corine Eising, Ton Groothuis
R.U.G., Groningen

SURVEILLANCE FOR AI IN POULTRY AND WILD BIRDS.

Ian Brown

CRL, VLA Weybridge, Surrey, United Kngdom

Implementation of surveys for avian influenza in poultry and wild birds in member states

Ian Brown

Community Reference Laboratory

Veterinary Laboratories Agency, UK

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

EMERGENCE OF HPAI

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

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

ITALY H7N1 Epidemic

LPAI (PEIPKGR*GLF)

(26.03.99-16.12.99)

HPAI (PEIPKGS**R**V**RR***GLF)

(17.12.99 - 05.04.2000)

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.''

Scientific committee on animal health and animal welfare

Recommendation IV

''Member States should put in place routine surveillance systems for the detection of influenza viruses in free-living birds''.

Scientific committee on animal health and animal welfare

Recommendation V

Member States should undertake serological surveys of poultry populations, especially those types of poultry reared or kept under conditions where contact with wild birds is likely, to determine the prevalence of infections with influenza A viruses of H5 and H7 subtypes, so that the potential economic impact of implementing Recommendation I (change of definition for avian influenza) can be assessed.

European seminar on pandemic planning in the Community

Conclusions

- Co-ordinated surveillance of influenza in humans and animals is needed.
- The human and veterinary surveillance systems should be linked to exchange information, diagnostic tools and antigens.

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

Implementation

- CRL to present/discuss guidelines for programmes at meeting of National Laboratories, Padova, June2002
- Approval of commission decision by SCoFCAH (July 2002)
- Member states to submit plan to commission for approval (mid October)
- Financial support for 50% of cost incurred
- Surveillance started before end of 2002

General structure of programme

- National Reference Laboratories
  - liaise with veterinary authorities for poultry survey
  - laboratory tests
  - submit H5/H7 viruses to CRL
  - collate results and submit CRL
  - organise and conduct wild bird survey

General structure of programme

- Community reference laboratory
  - technical support, protocols
  - reagents
  - functions as NRL
  - standard tests for H5/H7 viruses
  - data collation from NRL's
  - integration of veterinary and human networks for influenza surveillance

Detection of seroprevalence of H5/H7 in poultry except ducks and geese

Hosts

- Major hosts in member states
  - include fattening turkeys, chicken and turkey breeders, broilers, layers, farmed game birds, ratites
- Host susceptibility to influenza A virus
- Backyard flocks?

Sampling

- Sizes according to
  - density
- Each region (2000 km^2)
- Requirements
  - Identify at least 5% prevalence with 95% c.i
  - 95% probability of i/d positive birds assuming 30% seroprevalence in flock
  - 10 birds per farm

Samples and testing

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

Influenza A virus detection in poultry - ducks and geese

Ducks and geese survey

- Outdoor production if available
- Sampling size to ensure i/d of at least one infected flock if prevalence is at least 5% with 95 c.i
- 10 birds sampled per farm and tested as pools of 5
- Samples collected at abattoir?
- Cloacal swabs or faeces for virus isolation

Survey guidelines for wild birds

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

AVIAN INFLUENZA SITUATION IN HONG KONG

Prof. Ken Shortridge

Professor Shortridge first reviewed the AI situation in Hong Kong since 1997. HPAI due to H5N1 virus first occurred in poultry in Hong Kong on three chickens farms in March-May 1997 and then re-emerged in November. Surveillance of Hong Kong poultry markets in December 1997 indicated H5N1 infections were widespread, especially in chickens (19.5% isolation rate) but also in ducks (2.4%) and geese (2.5%). Control was established by slaughter of all poultry in Hong Kong, which was carried out between 29 December 1997 and 2 January 1998. HPAI H5N1 virus re-emerged in poultry in Hong Kong in 2001, but the virus was genetically distinguishable from the 1997 virus.

These HPAI outbreaks were given much greater importance and public awareness due to the human involvement. In May 1997 a virus of H5N1 subtype was isolated from a young child who died in Hong Kong and by December 1997 the same virus was confirmed by isolation to have infected 18 people, six of whom died. There was some evidence of very limited human-to-human spread of this virus, but clearly the efficiency of transmission was extremely low. The viruses isolated from the human cases appeared to be identical to viruses first isolated from chickens in Hong Kong in March 1997

He then described, from the laboratory point of view, the practicalities of dealing with a situation in which there was so much public concern and media interest.

Editor

MAIN ISSUES EMERGING FROM THE AI SYMPOSIUM IN ATHENS

Guus Koch

CIDC Lelystad, The Netherlands

Fifth International Symposium on Avian Influenza

- April 14-17, 2002
- Athens Georgia
- David Swayne ad Richard Slemons
- meeting every 5 years

WAGENINGEN UR

WHO

- **Scientist**
- **Veterinarians**
- **Biologist**
- **Government Regulators**

WAGENINGEN UR

MAIN ISSUES IN LAST 5 YEARS

- Transmission of H5N1 and H9N2 to humans
- role of LBM in the epidemiology of influenza in some countries
- lack of validation of tests (for instance Directogen®)
- reverse genetics system
- LPAI evolve to HPAI in chickens
  - struggle of politicians
  - lack of of solid contingency plans (lot of unsolved issues)

WAGENINGEN UR

What

- Global reports
- Avian Influenza Outbreaks in Italy and Hong Kong
- Avian Influenza Ecology and Epidemiology
- Advances in Molecular Biology and Molecular Epidemiology
- Impact on Public Health
- Pathology and Pathogenesis
- Risk Assessment, regulations and trade issues
- Field experiences in control and eradication
- Vaccines and antibody-based diagnostics
- Molecular diagnostics
- Late-braking issues

WAGENINGEN UR

Global reports

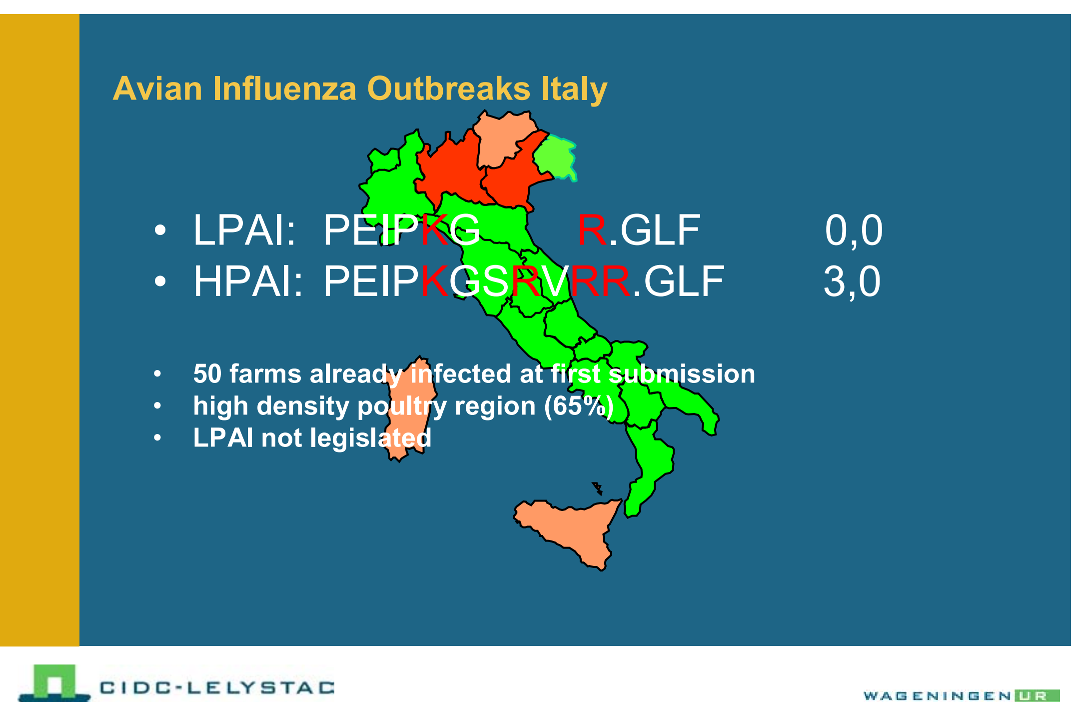
- Eastern hemisphere
  - HPAI: H5N2, H5N1, H7N1, H7N3
  - LPAI: H5N2, H5N9, H7N1, H7N7, H9N2 Endemic in Middle and Far East
  - Other subtypes quarantine, LBM
  - other subtypes death in IVPI; H10N7 (0,12)
- Western hemisphere
  - no HPAI last 5 years
  - HPAI H7N4in Australia RKRKR.G; IVPI increasing from 1,3 to 2,5
  - LPAI H5N2
  - LBM H7N2, since 94

WAGENINGEN UR

Avian Influenza Outbreaks Hong Kong

- Consumer preference for live poultry causing many problems
- LBM of Hong Kong still H5N1; i.e. re-assorted: internal genes similar to waterfowl
- depopulation, cleaning and disinfecting
- in 2001 rest day introduced quail separated
- in 2002 again H5N1 isolated
- parallel introduction of different genotypes of H5N1

WAGENINGEN UR



Avian Influenza Ecology and Epidemiology

- All subtypes in ducks and shorebirds
- influenza viruses evolve domestic chicken
- quail put forward as important intermediate host
- LBM in Nanchang mixing quail, turkeys and chickens
- all replicate in quail
- increased genotype diversity
- extensive re-assortments
- about 1% of wild birds: most subtypes represented
- new subtype form gull?

WAGENINGEN UR

Advances in Molecular Biology and Molecular Epidemiology
Impact on Public Health

- Reverse genetics
- mutant viruses:
  - M2(TMx): no ion channel function largely attenuated
    J. Virol. 75, 2001, 5658-5662
  - NS2(ko): no virus production abortive infection induces protection
    J. Virol. 76, 2002, 767-773
  - identification virulence factors: PB2
    - amino acid difference (K) in PB2 causes virulence in mice

WAGENINGEN UR

Impact on Public Health

- Influenza is risk factor for humans ('18-'19 pandemic)
- Meltzer J. Emerging Infect. Dis. 5 (1999) 659-671
  - 89.000-207.000 deaths and $71.3 to $166.5 billion costs.
- few cases of humans clinical signs form infections with avian strains.
- HK serological surveillance: Increased risk for people involved with poultry (Bridges J.ID, 185, 2002, 1005-1010)
  - 10% PW: H5-Antibodies
- HK:
  - 4.4% children pos. H9N2 Ab
  - 36% PW positive gs-ab, 23% spec. Testing
- little drift in avian sequence in 80 years

WAGENINGEN UR

Pathology and Pathogenesis

- Enhanced transmission of HPAI viruses compared to LPAI might select the former.
- Viruses acquire additional of glycosylation sites near receptor sites during passages in chickens
- HK virus can infect multiple avian species
  - clinical signs directly correlated to viral replication
  - heart, brain, pancreas most suitable for VI

WAGENINGEN UR

Risk Assessment, regulations and trade issues

- Accumulating evidence that HPAI evolve from LPAI asks for new legislation.
- Both in USA and EU considered as state responsibility
- H7N2 in Virginia controlled as HPAI. Compensation is industry responsibility
- LBM remain source of influenza virus
  - 60% of LBMs in NY and NJ positive for H7N2 and 18.8% of samples

WAGENINGEN UR

Field experiences in control and eradication

- HPAI in Mexico successful eradicated by using stamping out, enforcing biosecurity and use of vaccines
- in Italy vaccination was used to control LPAI. Although LPAI did re-emerged, of 23 farms infected only one was vaccinated.
- Information technology is indispensable. An advanced GIS system was developed and has been used in the Italy outbreak.
- Unsolved issues
  - survival of influenza in environment
  - inactivation of manure (week at ambient temp.; pH 2)
  - killing of poultry
  - large number of carcasses

WAGENINGEN UR

Vaccines and antibody-based diagnostics

- Recombinant H7 PMV-1 vaccine:
  - partial protective against ND and AI challenge
- production of antiserum by using DNA immunisation
  - less cross-reactive antisera for typing
- discriminatory test based on expression of heterologous N in insect cells
- Directogen® test used as on site test on swab samples.

WAGENINGEN UR

Molecular diagnostics

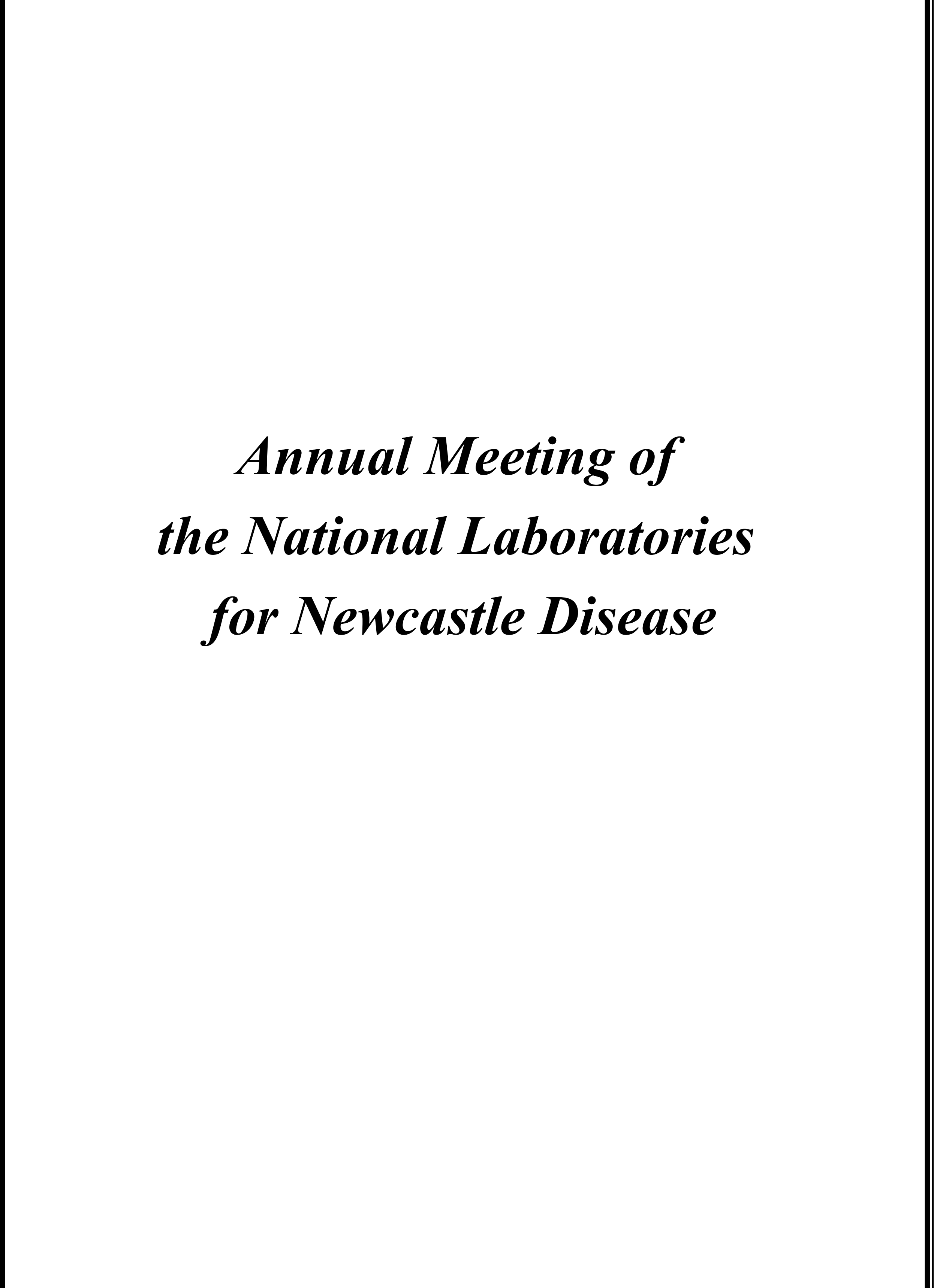
- RT-PCR
  - sensitivity 92% and specificity 97% using SPF eggs as standard
- H5 and H7 realtime RT-PCR
  - limited validation with LBM samples. Detected 93,8% of H7 VI+ and 96.5% H5 VI+.
- nucleic acid sequence-based amplification (NASBA) combined electrochemiluminescence.

WAGENINGEN UR

Late-braking issues

- Outbreak of LPAI in Virginia
  - confirmed after 6-7 days
  - decided after 10 days to control as HPAI outbreak
  - improvisations
- Outbreak of HPAI H5N1 in poultry farms Hong Kong
  - 3 genotypes involved
  - vaccination programme planned

WAGENINGEN UR



Annual Meeting of the National Laboratories for Newcastle Disease

TECHNICAL REPORT OF THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2001

Dennis J. Alexander and Ruth J. Manvell

Community Reference Laboratory for Avian Influenza
VLA-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom.

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 2001

(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 the basic amino acid 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 over the past 14 years 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***
605	*284*	*266*	*305*	*357*	*704*	*316*

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: 316 viruses were received during 2001 of which 163 were ND viruses see Table 2.

Table 2: Identification of viruses submitted to the CRL in 2001

Virus identification	Number
Influenza A viruses	***58***
Paramyxoviruses	***176***
APMV-1 [NDV]	163
APMV-2	5
APMV-3	7
APMV-6	1
others	***82***
reovirus	16
herpesvirus	12
flavivirus	2
circovirus	1
untyped	34
virus not viable	17

In addition to identification, 26 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: 70%

(2) Maintain a virus repository and stocks of reagents necessary for virus characterisation. Distribute viruses held in the repository and limited amounts of reagents to national laboratories on request.

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

***% Resources:** 12 %*

WORK DONE: The 163 ND 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:

ANTISERA :- 164 x 0.5ml of NDV antiserum was supplied; 11 x 0.5ml APMV-2 and 33 x 0.1ml of APMV-3 antiserum was also supplied.

ANTIGENS:- 1626 x 1.0ml of ND virus antigen, 3 x 1.0ml APMV-2 and 33 x 1.0ml APMV-3 antigen were supplied

Estimated actual % resources**: **10%

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		
NDV	50	8	100	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.*

(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:** 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**: **4%

(4) Analysis of results submitted by National Laboratories for the inter-laboratory comparison test.

***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 will be 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) Support by means of information and technical advice National Newcastle disease Laboratories and the European Commission during epidemics.

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

***% Resources:** 2 %*

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

Estimated actual % resources**: **2%

(7) Prepare programme and working documents for the Annual Meeting of National Newcastle Disease Laboratories to be held in 2001.

***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 Uppsala, Sweden in April 2001.

Estimated actual % resources**: **3%

(8) Collecting and editing of material for a report covering the annual meeting of National Newcastle Disease Laboratories to be held in Uppsala, April 2001.

***Work Plan**: Receive and collate submissions, edit and produce report of proceedings by end of 2001.*

***% Resources**: 4 %*
WORK DONE: Collation of the material and some editing has been undertaken, but at the end of 2001 the Proceedings had not been completed and published.

***Estimated actual % resources**: 4%*

(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 2001 relating to the work of CRL for ND.

RELEVANT PUBLICATIONS IN 2001

11. STANISLAWEK, W.L., MEERS, J., WILKS, C., HORNER, G.W., MORGAN C. & ALEXANDER, D.J. (2001). Survey for paramyxoviruses in caged birds, wild birds, and poultry in New Zealand. New Zealand Veterinary Journal 49, 18-23.
12. ALEXANDER, D.J (2001). Newcastle disease – The Gordon Memorial Lecture British Poultry Science 42, 5-22.
13. ALDOUS, E.W. & ALEXANDER, D.J. (2001). Technical Review: Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1) Avian Pathology, 30(2), 117-128
14. ALDOUS, E.W., COLLINS, M.S., MCGOLDRICK, A. & ALEXANDER, D.J. (2001). Rapid pathotyping of Newcastle disease virus (NDV) using fluorogenic probes in a PCR assay. Veterinary Microbiology 80, 201-213
15. ALEXANDER, D.J. (2000). Newcastle disease. Chapter 2.1.15. OIE Manual of Standards for diagnostic tests and vaccines. OIE : Paris pp 221-232
16. ALEXANDER, D.J. (2001) Paramyxoviridae - Paramyxovirinae. In Poultry diseases. F. Jordan. M. Pattison, D. Alexander & T. Faragher [eds]. W.B. Saunders, London pp 257-271.
17. CATTOLI, G., MANVELL, R. J., TISATO, T., BANKS, J. & CAPUA, I. (2001). Characterisation of Newcastle disease viruses isolated in Italy in 2000. Avian Pathology 30, 465-469.
18. HUOVILAINEN, A., EK-KOMMONEN, C., MANVELL, R.J. & KINNUNEN, L. (2001). Phylogenetic analysis of avian paramyxovirus 1 strains isolated in Finland. Archives of Virology 146, 1775-1785.
19. BAILEY, T., MANVELL, R.J., GOUGH, R.E., WERNERY, U. & KINNE, J. Review of Newcastle disease in bustards: Presentation, pathology and control; Results of vaccination trials. Proceedings of the 6th European AAV-DVG Conference, Munich, Germany. March 7 - 10, 2001 p202-207.

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

COUNTRY REPORTS ON NEWCASTLE DISEASE FOR 2001 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*

   If there were a large number of outbreaks i.e. in Italy give numbers of isolates with same properties and ranges of ICPI.

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*

***

A total of 29 questionnaires was sent to different laboratories. Responses were received for 14 laboratories of EU countries: Italy, Portugal, Spain, Greece, Ireland, UK, Northern Ireland, Denmark, Finland, France, The Netherlands, Sweden, Germany and Belgium, and 10 from non-EU countries: Slovenia, Norway, Cyprus, Estonia, Bulgaria, Poland, Slovak Republic, Latvia, Lithuania and Switzerland. The responses for number of samples processed for ND [APMV-1] are identical to those for avian influenza virus isolations [see above pages] the results in terms of avian paramyxovirus isolates are summarised in the following pages.

VIRUS ISOLATION REPORTS

BELGIUM/LUXEMBOURG

Bird	*Number*	*ICPI*	*amino acids*	*mAb group*	*conclusion*
Pigeon	7		^{113}RQKRF^{117}	P	PPMV-1
Psittacines	4				APMV-3
Small birds	7				APMV-3

BULGARIA

No isolates

CYPRUS

Bird	*Number*	*ICPI*	*amino acids*	*mAb group*	*conclusion*
psittacines	1				APMV-3

DENMARK

Bird	*ICPI*	*amino acids*	*mAb group*	*conclusion*
Pheasant	0.0	^{112}GRQGRL^{117}	nd[1]	Low virulent APMV-1
Ostrich	0.16	^{112}GKQGRL^{117}	nd	Low virulent APMV-1
Pheasant	-	-	PMV-2	PMV-2
Pheasant	0.25	^{112}EKQGRL^{117}	nd	Low virulent APMV-1
Cormorant[2]	1.44	^{112}RRQRRF^{117}	C1	High virulent APMV-1

[1]nd: not done [2]*Phalacrocorax carbo sinensis*

ESTONIA

No isolates.

FINLAND

No isolates

FRANCE

APMV-1 isolates

Bird	*Number*	*ICPI*	*amino acids*	*mAb group*	*conclusion*
Backyard chickens	1	0.3	nd		avirulent APMV-1
Meat turkeys	1	0.0	^{112}GRQGRL^{117}		avirulent APMV-1
Free pigeons	6	1.1-1.4		P	PPMV-1
Racing pigeons	1	1.4	nd	P	PPMV-1
Wild pigeons	1	1.4	nd	P	PPMV-1
Experimental sentinel ducks	8	nd	^{112}GKQGRL^{117}		avirulent APMV-1

Other APMVs

Bird	**Number**	**Type**
Meat turkeys	1	APMV-3
Budgerigar	1	APMV-3
Teals	1 1	APMV-4 APMV-6
Coots	1	APMV-6

GERMANY

Bird	*Number of isolates*	*Type*
Pigeons	22	APMV-1 (PPMV-1)
Chickens	6	APMV-1
Ducks	1	APMV-1
Ornamental chickens	2	APMV-1 (PPMV-1)
Parakeets	1	APMV-1 (PPMV-1)
Exotic pet birds	2	APMV-3
Greylag goose	1	APMV-1 (PPMV-1)

Characterization of APMV-1 isolates

Bird	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
pigeons	not tested	^{112}RRQKR∗F^{117}	P	PPMV-1
pigeons	not tested	^{112}RRKKR∗F^{117}	P	PPMV-1
chickens	not tested	^{112}GRQGR∗L^{117}	E	vaccine
duck	not tested	^{112}GRQGR∗L^{117}	E	vaccine
ornamental chickens	not tested	^{112}RRQKR∗F^{117}	P	PPMV-1
parakeet	not tested	^{112}RRQKR∗F^{117}	P	PPMV-1
Greylag goose	not tested		P	PPMV-1

GREECE

No isolates

IRELAND

No isolates

ITALY

Bird	*Number*	*ICPI*	*amino acid sequence*	*mAb group*	*Conclusion*
Broilers	6 1	0.2-1.2	not done	E P	6 x vaccine 1 x PPMV-1
Duck		0.2	not done	E	lentogenic APMV-1
Pigeons	31	0.77-1.3	^{112}GRQKRF^{117} ^{112}RRQKRF^{117}	P	31 x PPMV-1
Collared doves	34	0.6 -1.38	^{112}RRKKRF^{117} ^{112}RRQKRF^{117}	P	34 x PPMV-1
Rural chicken	1	1.9	^{112}RRQRRF^{11}	C1	velogenic APMV-1
Meat turkey	3	0.2-0.3	not done	E	3 x vaccine
Passerine (imported)	1				APMV-2
Psittacine	1				APMV-3

LATVIA

No isolates

LITHUANIA

No isolates

THE NETHERLANDS

No isolates

NORWAY

No isolates

POLAND

No isolates

PORTUGAL

Bird	*Number*	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
pigeon	1	not tested	^{112}GRQKR∗F^{117}	P	PPMV-1

SLOVAK REPUBLIC

No isolates.

SLOVENIA

Bird	*Number*	*ICPI*	*Amino acids*	*Conclusion*
pigeon	1	0.9	^{112}GRQKR∗F^{117}	PPMV-1

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

Bird	*Number*	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
pigeon	1	0.9		P	PPMV-1

UNITED KINGDOM [GREAT BRITAIN]

Bird	*Number*	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
pigeons	17	0.89 [1]		P	PPMV-1

UNITED KINGDOM [NORTHERN IRELAND]

Bird	*Number*	*ICPI*	*Amino acids*	*mAb group*	*Conclusion*
broilers	1	0.01		E	vaccine
pigeons	7	0.96-1.25		P	PPMV-1

Half of the 24 laboratories reported no isolation of avian paramyxoviruses. The other 12 laboratories reported a total of 186 avian paramyxoviruses. One hundred and sixty-six of these were APMV-1 viruses (Table 1). Thirty of these APMV-1 viruses were of low virulence representing the isolation of live vaccine viruses or naturally occurring avirulent viruses. Two virulent viruses showing C1 monoclonal antibody binding were reported, one obtained from rural chickens in Italy and the other isolated from a cormorant in Denmark. The latter is notable in view of the reported probable endemic presence of virulent APMV-1 in cormorants in North America. A total of 134 of the isolates was identified a APMV-1 viruses responsible for the ongoing panzootic in pigeons [PPMV-1]. Of these 65 were associated with the epidemic amongst pigeons and collared doves in Italy. However, PPMV-1 isolations were reported by 9 different laboratories and this emphasises the continued widespread presence of this virus in Europe and the continued threat this represents for domestic poultry and wild life. In three instances PPMV-1 was isolated from domestic poultry, from broiler breeders in Sweden, broilers in Italy and two isolated were obtained from ornamental chickens in Germany.

Table 1 Summary of APMV-1 virus isolations reported

Type of APMV-1	*Bird*	*Number*
PPMV-1	pigeons	95
	collared doves	34
	chickens	4
	psittacines	1
	goose	1
virulent APMV-1	backyard chickens	1 mAb C1
	cormorant	1 mAb C1
low virulence APMV-1	chickens	14
	ducks	10
	turkeys	4
	pheasants	2

SEROLOGY FOR ND

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

BULGARIA

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
fowl	14	340	0	0
ducks	10	274	1	20
geese	1	10	0	0
turkeys	1	30	0	0
pheasants	1	30	0	0

DENMARK

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
fowl	approx. 85	5206	0	0
aquatic birds		34	0	0
turkeys		63	0	0
ostriches		28	1	3[1]
pigeons		5	0	0
game birds	4-6	126	0	0
other species		18	0	0

[1] Subsequent virological examination gave a negative result

ESTONIA

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
Broilers	**27**	**3310**	**1**	**4**

FINLAND

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
layers	21	1686	0	0
broilers	45	5008	0	0
turkeys	26	1523	2 [imports]	51
ostriches	1	1	0	0
duck	1	20	0	0
geese	1	3	0	0
wild birds		4		0

NORWAY

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
Fowl	99	6742	2	92
Turkeys	7	441	0	0
Domestic ducks	8	379	0	0
Mallards	1	64	0	0

SWEDEN

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
breeders	118	6698	1	20
imported breeders	20	1709	0	0
turkeys	9	436	0	0
wild birds		215		0

SWITZERLAND

Type of poultry	*Number of flocks tested*	*Number of sera examined*	*Number of flocks positive*	*Number of sera positive*
laying hen	10	145	0	0
broiler	110	1762	0	0
turkey	17	330	0	0
pet bird		39		1 (APMV-3)
pigeon		1		1

The occasional detection of positive flocks, probably represents introduction of viruses of low virulence from infected feral birds. Virus isolation attempts on these flocks were usually negative and of the 7 countries only Sweden reported isolation of a PPMV-1 virus from a broiler breeder flock and Switzerland a PPMV-1 virus from a pigeon [see above].

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 20 years] remains a serious cause for concern and threat to poultry.

PIGEON PARAMYXOVIRUS TYPE-1 DISTRIBUTION IN ORGANS OF PIGEONS NATURALLY INFECTED WITH SALMONELLA TYPHIMURIUM, SSING A NP-BASED RT-NESTED PCR

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A NP-based RT-nested PCR to be applied for direct detection of type one pigeon paramyxovirus (pPMV-1) in organs, was previously described and validated on 120 organs by comparison with virus isolation in embryonated fowl eggs (Barbezange C. and Jestin V., J. Virol. Methods, 2002, 106, 197-207).
An experimental pPMV-1 infection was followed for 31 days after infection, using this NP-based RT-nested PCR, in 16 organs of oculo-nasally inoculated or contact pigeons naturally infected with *Salmonella* Typhimurium. With two exceptions, both groups presented similar results. Typical nervous signs and a green diarrhoea were observed. The spread of pPMV-1 was relatively quick, all organs being largely positive at 4 days after inoculation or contact. The lung, spleen, caecal tonsils, kidneys and brain, for which almost all tested samples remained positive during most of the experiment, seemed to be the principal targets for virus persistence. However, the virus was significantly recovered later in the brain parts and for longer in the trachea of the contact pigeons than of the inoculated ones (respectively, 7 days versus 4 days on one hand, and at least 28 days versus 14 days on the other hand) . Details are available in Barbezange C. and Jestin V., 2003 (Avian Path., 32, 275-281).
Such a method combining experimental mimic of the infection and molecular tool for virus detection, might be useful to assess vaccine efficacy or to better understand the pathogenesis of pPMV-1

infection.

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PPMV-1 EPIDEMIC IN ITALY

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Introduction

Italy has been experiencing an epidemic of Newcastle disease caused by the "pigeon strain" of avian paramyxovirus 1 (PPMV1), and a similar situation is occurring in other European countries (Alexander *et al.,*1998, Alexander *et al*. 1999b; Capua & Cancellotti, 1999). In 2001 the major part of PMV1 isolated in Italy were pigeon strains (see table 1).

Table 1: **Characterisation of APMV-1 isolated at the National Reference Laboratory for ND and AI of Italy in 2001**

type of bird	ICPI	mAb group	Conclusion
Broiler	0.2-1.2	E & P	6 x Vaccine 1 x PPMV-1
Duck	0.3	Not identifiable	1 x Lentogenic (vaccine ?)
Meat turkey	0.2-0.3	E	3 x Vaccine
Collared dove	0.68-1.38	P	34 x PPMV-1
Pigeon	0.77- 1.3	P	31 x PPMV-1

Materials and methods

During 2000 and 2001, a significant number of collared doves (*Streptopelia decaocto*) and feral pigeons (*Columba livia*) were collected from provinces of north–central Italy by the official veterinarians or by farmers and submitted for diagnostic investigations.

Selected organs (brain, lung and trachea and intestine) collected from dead or humanely killed moribund birds were processed for attempted virus isolation according to the guidelines indicated in the EU Council Directive 92/66/EEC (CEC, 1992). Haemagglutinating agents were identified by means of the haemagglutination inhibition test, using a monospecific antisera against NDV raised in SPF chickens, performed as indicated in EU directive 92/40/EEC (CEC, 1992).

Isolates were further classified by means of the HI test using monoclonal antibody 161/617 specific to pigeon-type PMV1 .

All isolates were tested for virulence by the intracerebral pathogenicity index (ICPI) test in day-old SPF chickens (CEC, 1992).

In order to determine the amino acid sequence of the fusion protein cleavage site, nucleic acid from the 20 dove and 12 pigeon isolates was extracted and a portion of approximately 400 bp of the fusion (F) gene was subjected to nucleotide sequencing, following previously described protocols (Collins *et al*., 1994; Alexander *et al*., 1999a). To detect any differences between isolates the variable F gene signal sequence was included in the amplicon. Sequences were then aligned together and phylogenetic analysis was performed for 305 nucleotides using the Maximum Likelihood Programme (DNAML) and neighbor-joining method (NEIGHBOR) from the PHYLIP phylogenetic inference package, version 3.5c (Felsenstein, 1993).

Results

All NDV isolates obtained from collared doves and pigeons reacted with mAb 161/617 that is specific for the "pigeon paramyxovirus 1" strain. The results of the ICPI tests performed indicate values ranging from 0.68 to 1.38. In addition, all the strains examined contain at the cleavage site of the fusion protein multiple basic amino acids at the C-terminus of the F2 protein and phenylalanine at residue 117, the N-terminus of the F1 protein, which is a typical feature of virulence. The phylogenetic tree is illustrated in Figure 1.

Phylogenetic analysis of the considered F gene sequences from PPMV1 isolates discerned three major genetic lineages (Figure 1): two lineages, marked I and II, contain isolates obtained from pigeons. They are phylogenetically clearly distinct as supported by significant bootstrap values. Lineage marked III, instead contained 18/20 of the isolates obtained from doves included in this study. Moreover, sequence analysis revealed 100% homology among the 18 isolates from doves present in lineage III. Homology between this lineage and isolates in lineage I was 98.4% and of 96.3% to the closest isolate 177/01 in lineage II.

The remaining two isolates obtained from doves, namely isolates 177/01 and 2736/V00 were placed in lineage I. It should be noted that among the isolates contained in this lineage there is a lower degree of homology among strains, when compared to the homology recorded for lineage III.

Discussion

No velogenic C1 viruses were obtained in 2001. PPMV1 is endemic in pigeon and collared dove population.

All ND strains isolated from collared doves and pigeons belong to the PPMV1 variant of Newcastle disease. These viruses was very virulent for doves and pigeons but have presented some difficulties in interpretation of the standard pathotyping test and although all isolates contained multiple basic amino acids at the deduced cleavage site of the F protein, ICPI values varied from 0.68 to 1.38. Variability in the ICPI values for PPMV1 has already been reported for isolates obtained from pigeons in Europe

(Meulemans *et al.,* 1998). The issue of having a genome sequence that is indicative of virulence, and an ICPI value below 0.7 (i.e. not notifiable) has been raised in the past and should be addressed in the future by competent organisations.

Phylogenetic analysis indicates that 18/20 dove isolates are virtually identical and cluster together, regardless of the geographical origin of the isolates. This appears to be a distinct lineage to the isolates obtained from feral pigeons in the same period. Nevertheless, low significant bootstrap value distinguishes this lineage from lineages I and II and a high level of homology was calculated between cluster I and III. Thus, it appears most likely that isolates from doves share a common ancestor from isolates in cluster I. Only two dove isolates, namely 177/01 and 2736/00, appear to fall out of this group, and cluster with pigeon isolates in lineage I.

The findings reported herein suggest that although collared doves and pigeons can share the same breeding and trophic niches, collared doves appear to be affected by a separate lineage of the PPMV1, and that spilling over of ND viruses from one population to the other occurs rather infrequently.

From an epidemiological point of view, it would be interesting to establish whether isolates obtained from collared doves in other countries also belong to a different lineage compared to the pigeon isolates of that country, and to establish how many isolates belonging to the "dove cluster" have been responsible of outbreaks in the domestic poultry population aiming to establish their role as reservoirs of PPMV1 viruses for the industrial poultry population.

Acknowledgements

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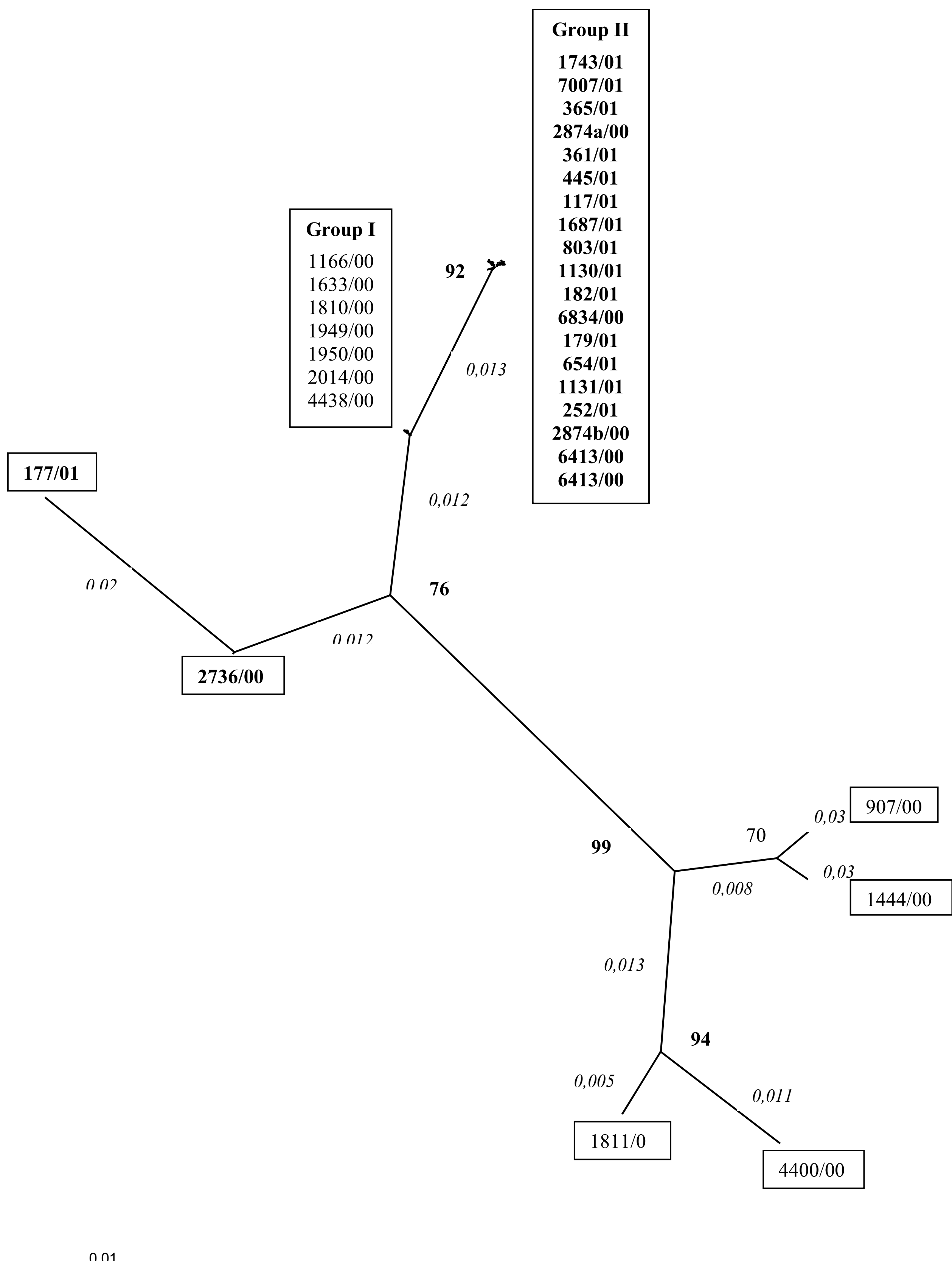

Fig. 1: Unrooted maximum likelihood phylogenetic tree based on 305 nucleotides of the F gene of 31 PPMV1 isolates from feral pigeons and doves (in bold). The branch lengths represent the predicted number of substitutions (values are indicated in italic) and are proportional to the differences between the isolates. Scale bar is indicated. Bootstrap values exceeding 70% are indicated in bold at the nodes.

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COMPARATIVE TESTS FOR ANTIGEN IDENTIFICATION AND HI TEST REPRODUCIBILITY IN DIFFERENT NATIONAL LABORATORIES 2002

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INTRODUCTION

One of the functions and duties of the Community Reference Laboratories for Newcastle Disease and Avian Influenza is to organise "periodical comparative tests in diagnostic procedures at Community level". To fulfil this duty a simple tests of the reproducibility in the National Laboratories of the haemagglutination inhibition [HI] test for the detection of Newcastle disease antibodies was organised in 1995 (Alexander 1996) and for H5 and H7 influenza virus antibodies in 1997 (Alexander, 1998). While tests of the ability of the National Laboratories to identify Newcastle disease and influenza virus antigens were organised in 1998, 1999 and 2001 (Alexander and Manvell, 1999, 2000 and 2002). At the 7th Annual Joint Meeting it was felt that while the antigen identification comparative tests were still revealing sufficient incorrect results to repeat the exercise, there was also a need for another test of the reproducibility of the HI test. It was decided therefore to send out 5 antigens for identification and six anti-APMV-1 sera.

The objectives of the tests were to be:

ANTIGEN IDENTIFICATION TEST
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.

HI TEST REPRODUCIBILITY
1. To compare the titres obtained in the different laboratories.
2. To identify problem areas.

As in the past results have been kept confidential to the submitting laboratory.

ANTIGEN IDENTIFICATION TEST RESULTS

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	A/turkey/Ontario/7732/66 (H5N9)	H5
B	APMV-1/chicken/Ulster/2C/67	APMV-1
C	APMV-3/turkey/England/1087/82	APMV-3
D	A/ostrich/Denmark/72420/96 (H5N2)	H5
E	A/African starling/EnglandQ/983/79 (H7N1)	H7

RESULTS

General

Twenty-nine 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 13 non-EU states participated these were: Bulgaria, Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Norway, Poland, Romania, Slovak Republic, Slovenia and Switzerland.

In total 140 results were received from the 29 laboratories. The correct results were obtained on 120 [85.7%] occasions. Results judged not to be wholly correct without actually being wrong [lesser-shaded cells in Tables 1 and 2] were given on only one [0.7%] occasion. Nineteen [14.0%] were wrong either because they failed to identify APMV-1, H5 or H7 antigens, or because they identified the APMV-3 virus as APMV-1 [see 24 in Table 1 and 8, 10, 20, 22 & 23 in Table 2]

Of the 29 participating laboratories, 17 fully identified all HA antigens. Eight laboratories had one unacceptable result and 4 had more than one unacceptable result.

Results by antigen

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

Nineteen laboratories identified this antigen correctly as H5. Eight of these laboratories volunteered additional identification of the neuraminidase, two of these laboratories, 8 and 12, incorrectly identified the neuraminidase as N2. Seven laboratories produced an incorrect result. Four of these identified the virus and influenza, but did not identify subtype, one identified it as H7 and two as APMV-3. Three laboratories reported no HA activity or an empty vial.

ANTIGEN B – virus APMV-1/chicken/Ulster/2C/67 – correct result APMV-1.

APMV-1/chicken/Ulster/2C/67 is the virus recommended for use as the standard antigen in haemagglutination inhibition tests in the EU, identification as APMV-1 should therefore have been straightforward. Only one laboratory failed to give the correct result [13] identifying the antigen as an H5 influenza virus.

ANTIGEN C – 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] that all laboratories should hold APMV-3 antiserum to enable identification.

Twenty of the 27 laboratories submitting a result identified antigen C as APMV-3. One laboratory reported APMV-?, but not APMV-1 [15, Table 2]. Six laboratories fell into the cross reaction trap and reported the virus as APMV-1. Two laboratories failed to detect HA activity in their vials.

ANTIGEN D – virus A/ostrich/Denmark/72420/96 (H5N2) – correct result H5.

Twenty-five of the 29 laboratories reported the correct identification of this virus as H5 and 9 of these offered the additional correct information that the neuraminidase was N2. The four laboratories with incorrect results reported either ‘influenza of undetermined subtype’ or H9, the latter possibly because the N2 antigen caused cross reaction in the HI tests with their H9N2 antiserum.

ANTIGEN E – virus A/African starling/England-Q/983/79 (H7N1) – correct result H7.

This was the only antigen for which all 29 laboratories gave the correct result. Eleven laboratories gave the additional correct information that the neuraminidase was N1, although one laboratory incorrectly identified it as N7.

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 2002 25 had taken part in 2000. The comparative results for the two years were:

Number that:-

	2000	2002
Satisfactorily identified all antigens:	16	17
Had one unacceptable result	3	5
Had more than one wrong	6	3

In fact 8 laboratories showed an improvement; 13 were the same with all results correct; one was the same with two incorrect results and 3 laboratories obtained worse results than 2000. No country fell into any other possible category.

Table 1. Results of Comparative Antigen Identification Tests

EUROPEAN UNION COUNTRIES

	ANTIGENS				
Country code	**A**	**B**	**C**	**D**	**E**
CRL	H5N9	APMV-1	APMV-3	H5N2	H7N1
1	H5	APMV-1	APMV-3	H5	H7
3	H5	APMV-1	APMV-3	H5	H7
4	H5	APMV-1	APMV-3	H5	H7
6	H5 (N9?)	APMV-1	APMV-3	H5(N2?)	H7(N1?)
7	H5N9	APMV-1	APMV-3	H5N2	H7N1
11	H5	APMV-1	APMV-3	H5	H7
14	H5	APMV-1	APMV-3	H5	H7
16	H5	APMV-1	APMV-3	H5	H7
17	H5N9	APMV-1	APMV-3	H5N2	H7N1
18	H5N9	APMV-1	APMV-3	H5N2	H7
21	H5	APMV-1	APMV-3	H5	H7
24	**APMV-3**	APMV-1	**APMV-1**	H5	H7
25	H5	APMV-1	APMV-3	H5	H7
26	H5N9	APMV-1	APMV-3	H5	H7N1
27	H5N9	APMV-1	APMV-3	H5N2	H7N1
28	H5	APMV-1	APMV-3	H5	H7

NON-EU COUNTRIES

	ANTIGENS				
Country code	**A**	**B**	**C**	**D**	**E**
CRL	H5N9	APMV-1	APMV-3	H5N2	H7N1
2	No HA	APMV-1	APMV-3	**H9**	H7
5	H5	APMV-1	**APMV-1or3**	H5	H7
8	H5**N2**	APMV-1	APMV-3	**Flu?**	H7N1
9	**APMV-3**	APMV-1	APMV-3	H5	H7
10	?IS	APMV-1	**APMV-1**	H5N2	H7N1
12	H5**N2**	APMV-1	No HA	H5N2	H7**N7**
13	**H7**	**H5**	**Flu H?**	**H9?**	H7
15	**Flu**	APMV-1	**APMV-?**	H5	H7
19	**Flu**	APMV-1	APMV-3	H5N2	H7N1
20	**Flu**	APMV-1	**APMV-1**	H5	H7
22	Vial empty	APMV-1	**APMV-1**	H5N2	H7N1
23	**Flu not 5/7**	APMV-1	**APMV-1**	H5	H7
29	H5	APMV-1	no HA	**H5?H7?**	H7

APMV-1 HI TEST REPRODUCIBILITY RESULTS

Table 2. Results of comparative tests for APMV-1 HI antibodies

EUROPEAN UNION COUNTRIES

Country Code	Sera					
	2/97	**5/88**	**1/02**	**6/76**	**1/87**	**Goat**
1	512	64	128	256	128	128
3	1024	256	256	256	2048	128
4	1024	128	128	256	256	128
6	1024	256	256	512	512	1024
7	1024	128	256	512	256	256
11	1024	128	256	256	512	128
14	2048	256	512	512	512	1024
16	256	64	64	-	128	-
17	512	128	64	1024	256	64
18	1024	256	256	512	512	256
21	512	128	128	256	256	128
24	1024	64	1024	Neg	512	Neg
25	512	256	256	256	128	128
26	128	32	32	Neg	32	32
27	1024	128	256	512	256	128
28	512	128	128	n.d	256	64

NON-EU COUNTRIES

Country Code	Sera					
	2/97	**5/88**	**1/02**	**6/76**	**1/87**	**Goat**
2	362*	>512	512	>32	724*	724*
5	1024	512	512	512	512	2048
8	1024	128	128	512	256	128
9	2048	128	256	Neg	512	1024
10	512	128	256	16	256	512
12	2048	1024	512	1024	512	256
13	1024	128	256	512	512	128
15	512	512	512	128	512	1024
19	512	256	256	128	256	128
20	256	64	64	Neg	128	64
22	1024	128	128	Neg.	128	256
23	512	256	512	512	512	512
29	1024	256	362	Neg	362*	362*

*geometric mean titres where range given

DISCUSSION

Assessment in performance of a laboratory in comparative HI titre tests in not straightforward. The various statistical analyses that may be used do not seem to be particularly applicable in the present situation. However, to some extent performance is prescribed in Directive 92/66/EEC as Annex III chapter 6 (j) states for HI tests that "The validity of the results is dependent on obtaining....................a titre within one dilution of the known titre of the positive control serum." If this is extrapolated to the current comparative tests a rough guide to the accuracy of testing in a given laboratory would be if the titre obtained with an antiserum sample is within one dilution of the "known" titre, which is probably best interpreted as the consensus titre of all the laboratories. The results for all laboratories are summarised in Table 3.

Table 3. Summary of the comparative HI results

HI titre	Number of laboratories with titre to serum					
	2/97	**5/88**	**1/02**	**6/76**	**1/87**	**Goat**
<2				6		2
16				1		
32		1	1		1	1
64		4	3			3
128	1	12	6	2	5	10
256	2	8	12	6	10	5
512	10	2	6	9	12	2
1024	13	2	1	2		5
2048	3				1	1
not done				3		

Of 171 HI results reported from all laboratories 35 [20.5%] were outside these limits 21 were too low and 14 too high. Laboratories that consider they performed badly in these tests should review their HI test procedure and ensure that it conforms to the protocol specified in Directive 92/66/EEC. It is particularly important that Ulster 2C antigen should be used.

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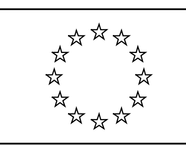

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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

SANCO/10057/2002

EC contribution to the Annual meeting of the National Laboratories for Newcastle disease and avian influenza

Held on 20-21 June 2002 at the
Istituto Sperimentale Zooprofilattico delle Venezie,
Legnaro -Padova-Italy

COMMISSION DOCUMENT
Avian influenza and Newcastle disease in the European Union in 2001-2002
Legislative aspects -Document SANCO/10057/2002

Maria Pittman
European Commission, Directorate General for Health and Consumer Protection

DISEASE NOTIFICATION AND SITUATION

Outbreaks of avian influenza and Newcastle disease reported by the Member States through the ADNS (Animal Disease Notification System) in the years 1998-2002 are shown in the following tables and graphs.

Avian influenza

Table 1: Number of outbreaks of avian influenza reported by Member States according to the ADNS system.

During the reporting period between April 2001-June 2002 there were no outbreaks of highly pathogenic avian influenza reported.

	1998	1999	2000	2001	2002
Austria	0	0	0	0	0
Belgium	0	0	0	0	0
Denmark	0	0	0	0	0
Finland	0	0	0	0	0
France	0	0	0	0	0
Germany	0	0	0	0	0
Greece	0	0	0	0	0
Ireland	0	0	0	0	0
Italy	1	58	351	0	0
Luxembourg	0	0	0	0	0
Netherlands	0	0	0	0	0
Portugal	0	0	0	0	0
Spain	0	0	0	0	0
Sweden	0	0	0	0	0
U.K.	0	0	0	0	0
TOTAL	**1**	**58**	**351**	**0**	**0**

Newcastle disease

Table 2: Number of outbreaks of Newcastle disease reported by the Member States according to the ADNS system from 1998-2002.

During the reporting period of April 2001 - June 2002 there were no outbreaks of Newcastle disease reported.

COUNTRY	1998	1999	2000	2001	2002
Austria	6	4	0	0	0
Belgium	14	0	0	0	0
Denmark	2	0	0	0	0
Finland	0	0	0	0	0
France	1	1	0	0	0
Germany	0	0	0	0	0
Greece	0	0	0	0	0
Ireland	0	0	0	0	0
Italy	2	1	256	1	0
Luxembourg	0	0	0	0	0
Netherlands	0	1	0	0	0
Portugal	0	0	0	0	0
Spain	0	0	0	0	0
Sweden	0	0	0	1	0
United Kingdom	0	0	0	0	0
TOTAL	**25**	**7**	**257**	**2**	**0**

VELOGENIC AVIAN PARAMYXOVIRUS-1 IN WILD BIRDS

Denmark

Denmark has reported positive findings ofAvian Paramyxovirus -1 with an ICPI> 0.7 in pheasants found dead in the north-western part of Sjælland in October 2000. The birds had been bought about 3 months before and immediately released. Poultry holdings in the surrounding area were clinically and serologically controlled and found negative as well as the holding of origin.

In November 2001 a velogenic Avian Paramyxovirus 1 with an ICPI of 1.44 was detected in a cormorant in western Sjælland. The wild bird had been shot for scientific purposes and showed no signs of illness.

Note that Community legislation on Newcastle disease does not apply for free living birds. However, when the disease is detected the Member State concerned shall inform the Commission of any measures it takes.

LEGISLATION IN RESPECT TO AVIAN INFLUENZA IN ITALY

Background:
In 1999/2000 Italy was affected by the most severe epidemic of AI ever to occur in Europe with 413 outbreaks causing the death of 16 Million heads of poultry. It is most likely that the mutation of a low pathogenic virus into a highly pathogenic one was responsible for this epidemic. In absence of a legislative basis in Community law to combat low pathogenic viruses, the disease situation was complicated.

The last outbreak of highly pathogenic avian influenza was detected on 5 April 2000. Since then Italy has experienced further occurrence of low pathogenic avian influenza infections. These were brought under control rather rigorously by the Italian authorities but the authorities also called for an emergency vaccination programme in order to effectively support the control measures. Since November 2000, a vaccination programme, approved by Commission Decision was carried out in a very small confined area of Verona province in the Veneto region. Based on the results of ongoing surveillance in and outside the vaccination zone, it was concluded that the virus was not circulating any longer and it was decided to phase out vaccination by May 2002.

Commission Decisions:
Commission Decision 2000/721/EC of 7 November 2000 introduced vaccination to supplement the measures to control avian influenza in Italy and on specific control measures.

In the light of the disease evolution, the following amendments were made to the original Decision:

Commission Decision 2000/785/EC of 12 December 2000 in order to "release" hatching eggs and day-old chicks from certain areas, where no vaccination was carried out and to extend trade restrictions to table eggs originating from the vaccination area.

Commission Decision 2001/627/EC of 11 August 2001 limiting the trade restrictions only to products originating from vaccinated birds and not to all products from birds kept in the restricted area.

Commission Decision 2001/847/EC of 1 December 2001 approving the iIFA-test that enables differentiation between vaccinated and infected turkeys and to authorise intra-Community trade of meat from vaccinated turkeys under a certain testing regime.

Commission Decision 2002/551/EC repealing the original Decision 2000/721/EC and **Commission Decision 2002/552/EC** of 9 July 2002 laying down which restrictive measures related to vaccination against avian influenza in Italy shall be maintained after the end of vaccination. Certain prohibitions for intra-Community trade in vaccinated live birds and products deriving from vaccinated birds stay in place and further monitoring of vaccinated flocks for a period of six months has to be carried out.

LEGISLATION AS A CONSEQUENCE TO OUTBREAKS OF AI/ND

Contingency plans for avian influenza and Newcastle disease

By **Commission Decisions 2000/680/EC and 2001/525/EC,** the contingency plans for avian influenza and Newcastle disease were approved. These had been drawn up by Member States' authorities according the Community guidelines and were presented during meetings held in Brussels.

EU legislation contains in Council Directives 92/40/EEC and 92/66/EEC provisions for the establishment of contingency plans for avian influenza and Newcastle disease. The objective of these plans is to enhance the preparedness of the Member States to encounter outbreaks of Avian Influenza and Newcastle Disease. Besides the necessary legal and financial powers, a well-structured administration, the prompt availability of equipment and manpower to deal with an epidemic the Member States must ensure that a full-equipped national laboratory with expert personnel is designated for the specific disease to guarantee quick and reliable diagnosis.

Information on the use of ND-vaccines in intra-Community trade

Commission Decision 2001/867/EC amending the Annex of Council Directive 90/539/EEC in order to introduce obligatory indication of information on the date of vaccination and the type of vaccine used against Newcastle disease in the certificates used for intra-Community trade of hatching eggs and live poultry.

SAFEGUARD MEASURES IN RELATION TO THIRD COUNTRIES

Avian influenza in Hong Kong

In May 2001 avian influenza of subtype H5N1 was re-occurring in Hong-Kong and affected several poultry holdings. Although transmission to humans was not reported and the European Union has no trade in live poultry or poultry meat **Commission Decision 2001/495/EC** was adopted for imports of birds from Hong Kong, Macao and China**.**

Newcastle disease in Australia

An outbreak of Newcastle disease occurred in May 2002 in a layer farm in the State of Victoria. It appeared that subsequently epidemiological links could be established to earlier outbreaks of ND in the State of New South Wales.

Commission Decision 2002/537/EC was adopted by banning all importation of live poultry, poultry meat, poultry meat products and poultry meat preparations from the territory of Australia.

Australia's main interest for trade with the European Union is ratite meat. By this Decision a certificate for ratite meat with similar guarantees as in the one used for the imports of ratite meat from the South African States was introduced requiring virus isolation tests with negative results for PMV-1 greater than 0,4 from each slaughter flock.

IMPORTATION OF LIVE RATITES

Commission Decision 2001/751/EC on the importation of live ratites established the animal health guarantees for the importation of live ratites from third Countries and a list of countries from where the importation into the Community is authorised. Imports can also take place from countries considered as not ND-free under the following conditions:

- Routine ND Surveillance on ratite farms following a statistically based sampling plan
- Prior dispatch 21 days quarantine - virological testing for ND (no PMV1 with ICPI>0,4)
- After importation quarantine/virus isolation test for ND

In relation to **Crimean Congo Haemorrhagic Fever (CCHF**) the following provisions have to be applied if the ratites originate from Asia or Africa:

In the country of origin:

- Isolation in tick proofed surroundings with an officially approved programme for rodent control for at least 21 days prior to export
- Anti-tick treatment before entering the isolation facility
- After 14 days of isolation birds have to be subjected to the competitive ELISA for antibodies against CCHF giving a negative result.

After importation into the EU:

- After arrival anti parasitic treatment
- After 14 days competitive ELISA test on each ratite
- Positive birds must be destroyed
- Re-testing of contact birds 21 days after first sampling, destroy whole group if one positive

The Food and Veterinary Office (FVO) has carried out missions to Botswana, Namibia, South Africa and to Zimbabwe in order to assess the situation on the spot. Reports are available on the web site of the Commission:

http://europa.eu.int/comm/food/fs/inspections/vi/reports/index_en.html.

IMPORTATION OF LIVE POULTRY FOR BREEDING AND PRODUCTION

Rules for the importation of hatching eggs, day old chicks, breeding and productive poultry and for restocking supplies of wild game are laid down in **Commission Decision 96/482/EC**. Problems have been encountered mainly in relation to imports of game for restocking. However, it was felt that compulsory testing for AI/ND by reducing the isolation period would be an appropriate alternative to the current procedure of:

- Separation for at least 6 weeks

- Clinical inspection by the Official Veterinarian

Amendment of Decision 96/482/EC by **Commission Decision 2002/542/EC** allows alternative procedure:

- Separation for 3 weeks
- Virus isolation tests for AI/ND on 60 birds

IMPORTATION OF SPECIFIED PATHOGEN FREE (SPF) EGGS

Commission Decision 2001/393/EC has established a certificate for the importation of SPF eggs. SPF-eggs were defined as hatching eggs used for diagnostic procedures in laboratories, vaccine production, research and for pharmaceutical purposes. They must be produced in accordance with the European Pharmacopoeia and must be destroyed after use or if not used. Importation is possible from all countries listed in part I of Annex to Council Dec.79/542/EEC. This is a very broad list of countries from where importation of products of animal origin takes place and where veterinary certification appears to be reliable. It was deemed that if the strict provisions of the European Pharmacopoeia can be fulfilled, the disease status of a country is not relevant.

FURTHER DECISIONS IN RESPECT OF THIRD COUNTRIES

By the following Commission Decisions several Third Countries were granted access to the European egg or poultry market or less stringent requirements were imposed for certain imports. Their disease situation, the guarantees they can offer in relation to animal and public health and the control system they have in place to ensure compliance with Community requirements were verified by evaluation of received written documentation and by the FVO reports.

2001/299/EC: ***Iceland*** - authorisation for table eggs

2001/598/EC: ***Brazil, Thailand and Croatia***
- amendments to authorised regions and less restrictive sanitary conditions in relation to NCD for fresh poultry meat
- consolidation of several Decisions for poultry meat imports from one specific country

2001/184/EC, 2001/732/EC, 2001/733/EC: ***Latvia*** - Authorisation for poultry meat products exports to the EU and inclusion on the country "list of principle" for live poultry and fresh poultry meat fulfilling the prerequisites for being authorised when a few still outstanding guarantees are provided for listing on the "harmonised lists".

2001/793/EC, 2001/794/EC: ***Argentina, Thailand, Tunisia*** - due to positive developments in the control of OIE list A poultry diseases requirements for wild bird game meat, farmed bird game meat and poultry meat products could be altered accordingly

2001/659/EC: ***Brazil***-was granted equivalency for their measures to deal with ND outbreaks

2001/183/EC: ***Bulgaria*** was authorised for live poultry imports into the EU
EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

Regulation (EC) No 178/2002 of 28 January 2002 has established EFSA, which was a key priority of the "White paper on Food Safety" 2000. It aims at a high level of protection of human health and shall enhance the "Farm to fork"- "Table to Stable" approach. The independent authority shall provide scientific advice and technical support for the Community's legislation and policies. It shall carry out risk assessments and shall be responsible for accurate, independent information and risk communication. The current Scientific Committees will undergo reorganisation under the patronage of EFSA. Currently EFSA has taken up its activities in Brussels, but different locations in other Member States like Helsinki (Finland), Parma (Italy) and Barcelona (Spain) are in discussion for housing EFSA.

WORK PROGRAMME FOR 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 FO protein in the virus and phylogenetic analysis

    c) Antigenic grouping of viruses

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.

WORK PROGRAMME FOR 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.

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 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 results of the surveillance programmes for influenza and other scientific information available:

   - keep under review the possible zoonotic impact arising from the risk of reassortment between influenza viruses,

   - perform a risk/benefit analysis on the eradication of influenza subtypes H5 and H7 from the poultry population.

10. Assist in the preparation and co-ordinate surveillance programmes on influenza in poultry and wild birds to be implemented in the Member States in 2002.

11. Co-ordinate and assist the monitoring and surveillance activities in the Member States concerning birds to estimate prevalence of influenza viruses and possibly to identify risks for humans.

12. Evaluate the results obtained by the Member States' surveys.

13. Liaise and exchange information with EISS (European Influenza Surveillance System) and WHO as regards the zoonotic aspect of influenza infections in animals with a view to co-ordinating methods and data collected for influenza surveillance in humans and animals.

14. To keep an enlarged stock of diagnostic reagents available to supply to other laboratories at the request of the Commission, e.g. in case of serious influenza epidemics in animals or humans, and to link with laboratories for surveillance in humans in order to develop an integrated quality assurance system, including sharing of diagnostic reagents.

15. Co-operate with other scientific institutions to verify the needs to extend the surveillance programme under point 10 above to species other than birds such as pigs involved in the transmission of influenza viruses to humans.

16. 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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LABORATORY ISSUES

ISSUES RAISED

1. Possible ring tests for QC of virus isolation techniques. [raised by *Gyorgy Czifra* (SE)]

Discussion at the meeting reached general consensus that comparative tests for virus isolation were impracticable. The reasons put forward were primarily:

a) It would be inappropriate to send virulent viruses and not really desirable to send viruses of low virulence.
b) Such tests would be more a measure of the freeze drying, storage and delivery processes than the virus isolation techniques.
c) Comparative tests would not measure the ability of the laboratory to isolate virus, as under field conditions the most important factors are the storage and treatment of samples rather than the actual inoculation into embryos, but the latter is the best you can hope to measure in comparative tests.

2. Validation of RT-PCR for detection of AI and ND [raised by *Guus Koch* (NL)]

Guus Koch raised the issue of validating RT-PCR tests for the detection of AI and ND as these were being used more routinely in a wider number of National Laboratories. He showed the results obtained in his laboratory for PCR on cloacal and tracheal swabs for NDV compared to virus isolation. These results [see pages 114-117] demonstrated that while RT-PCR showed high specificity relative to virus isolation for both types of swabs, it had low sensitivity if cloacal swabs were used.

There was also discussion on the similarity of PCR tests in different laboratories and the nature any “ring test” would take. The CRL undertook to consider this and implement an interlaboratory test of some description in 2004, if not sooner.

3. Comparison of sensitivity of virus isolation using 3 days instead of 6 days of incubation (preliminary data) [raised by *Guus Koch* (NL)]

The general agreement was that two passages of 6 days as prescribed in Directives 92/40/EEC and 92/66/EEC results in unacceptable delay in declaring a negative result, which in the case of broiler chickens may result in serious welfare problems. Guus Koch presented some preliminary data showing similar isolation rates if only 3 days incubation was used for two passages. UK representatives reported using a slightly different technique of re-passaging half the inoculated eggs at day three and allowing those remaining to be incubated to day 6, when all the eggs were examined for haemagglutinating agents. It was agreed to continue these studies, before making a decisive statement at the next annual meeting.

4. Identification of AIV: how to be sure that there is not mixed isolates when we observe cross reactivity with other subtypes? [raised by *Veronique Jestin* (F)]

It was agreed that mixed isolates do represent a significant challenge in diagnosis, and this will vary with the ratio and nature of the two viruses. Usually when the viruses are not high and low virulence strains of the same haemagglutinin group the problem is of failure to detect any or full inhibition of a haemagglutinating agent with the reference antisera, which can usually be resolved by re-growing the viruses after treatment with the most likely serum against one of the viruses. However, at present, using conventional techniques there is no easy solution and identifying both viruses is irksome and time-consuming. Hopefully, molecular techniques will be developed that simplify this process.

5. Accurate/sure identification of APMV-3 isolates if there is no more Mab 50 (APMV-3 specific) available or a substitute [raised by *Veronique Jestin* (F)]

The loss of the hybridoma for mab 50 does represent a significant problem for which there is currently no obvious solution.

6. Non specific HI responses: what is the best method to reduce them in species other than *Gallus gallus* (kaolin, hemadsorption : in this case use of homologous red blood cells or not?....)? [raised by *Jean Paul Picault* (F)]

The consensus was that receptor destroying enzyme (RDE) treatment, possibly with heat inactivation at 56°C for 30 minutes was probably the best approach for non-specific inhibition. Kaolin treatment should be avoided. Heterologous red blood cells more often resulted in non-specific agglutination which could be removed by first treating the sera with the RBCs to be used in the HI tezst.

7. advice on the quality (specificity....) of AI commercial ELISA kits [raised by *Jean Paul Picault* (F)]

The issue was discussed. Some reported high levels of false positives with some ELISA kits others using them had not observed this. It was proposed to review this again next year.

8. tissue culture versus egg inoculation for PPMV-1 isolation. [raised by *Dennis Alexander* (UK)]

This matter followed on from previous meetings in which it was considered that some PPMV-1 viruses did not appear to grow well in eggs, especially at 1st passage, and that chick embryo liver (CEL) cells appeared to perform better.

PPMV-1 isolations in chick embryos and CEL cells at VLA Weybridge during 2001-2002 were: 71 pigeons received suspected of PPMV-1 infection from clinical signs. Infection confirmed by virus isolation in 28, isolated as follows.

eggs 1st	eggs only	CEL 1st	CEL only	both same time
8	3	1	2	14

Thus although eggs (chick embryos) out performed CEL cells, there were two occasions when viruses were isolated only in CEL cells, confirming the usefulness of using both methods.

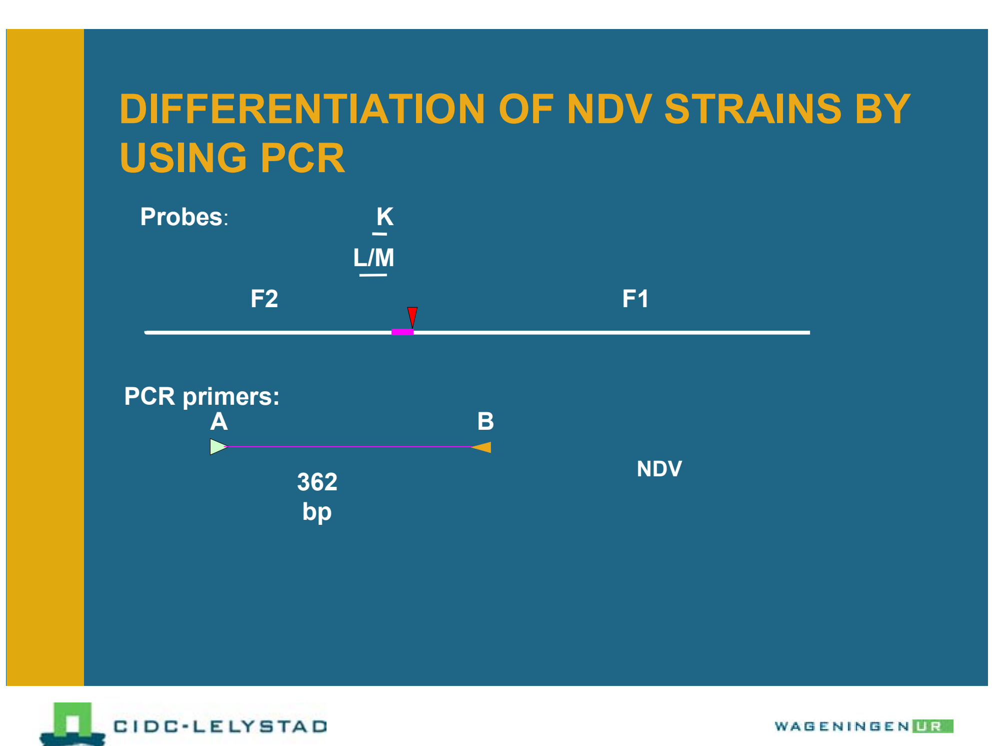
ASSESSMENT OF THE SENSITIVITY AND SPECIFICITY OF PCR FOR NDV ON CLOACAL AND TRACHEAL SWABS COMPARED TO VIRUS ISOLATION.

Guus Koch, CIDC Lelystad, The Netherlands

EXPERIMENTAL SETUP

- 15 WLA SPF chicken 4 weeks of age
  - 10 infected i.n./i.o. with 10^6 EID_{50} Carlifornia strain NDV
  - 5 inoculated with normal allantoic fluid
  - kept in 2 seperate isolators
- Daily treachea swab and trachea swab during 14 days
- 200 ul used for virus isolation, and 100 ul for RNA isolation

CIDC-LELYSTAD WAGENINGEN UR



COMPARISON OF PCR WITH VIRUS ISOLATION ON CLOACA SWABS

RT-PCR	Virus isolation	
	+	-
+	13	0
-	32	36

Sensitivity	28,9%
Specifity	100%
Predictive value	100%

WAGENINGEN UR

COMPARISON OF PCR WITH VIRUS ISOLATION ON TRACHEA SWABS

RT-PCR	Virus isolation	
	+	-
+	45	2
-	11	22

Sensitivity	80,4%
Specifity	91,7%
Predictive value	95,4%

WAGENINGEN UR

Validation Newcastle disease virus RT-PCR
367.47105-00/2000.27

<u>Source</u>: **trachea** swabs

Chick number	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	Day 13	day 14
1	-++/+		+++/+				+++/+	-+-/+	-+-/+	---/-	--+/-	---/-		
2	++-/-	+++/+	+++/+	+++/+	+++/+	+++/+	+++/+	+++/+	+-+/+	-++/-	+-a/+	+-+/-	---	---/-
3	+++/+		+++/+		†	†	†	†	†	†	†	†	†	†
4	+-+/+	/+	+++/+	+++/+	/+	/+	+*+/+	+++/+	+++/+	-+-/-	---/+	---/+	/-	
5	++-/+		+++/+				+++/-	+++/+	++-/+	---/-		---/-		
6	+++/-		+++/+				+++/+	+++/-	+++/+	---/-		---/-		
7	-++/+		-++/+		†	†	†	†	†	†	†	†	†	†
8	+++/-		+++/+				+++/+	+++/+	+++/+	-++/-	+--/+	---		
9	+++/+		+++/+	+++/+	†	†	†	†	†	†	†	†	†	†
10	+++/+		+++/+				+++/+	+++/+	a++/+	++-/-	---	---		
11	---/-		nd/-	---/-			nd/-	---/-						
12	---/-		nd/-	---/-			nd/-	---/-						
13	---/-		nd/-	---/-			nd/-	---/-						
14	---/-		nd/-	---/-			nd/-	---/-						
15	---/-		nd/-	---/-			nd/-	---/-						

<u>Notation:</u> egg 1, egg 2, egg 3 / PCR result

*: Allantoic fluid not suitable for HA test.
nd: not done
a: non-specific sample (embryo died within 24 h , not tested.
[black] PCR is positive, virus isolation negative
[dark grey] Both PCR and VI positive
[light grey] Virus isolation positive PCR negative

Validation Newcastle disease virus RT-PCR
367.47105-00/2000.27

source: **cloaca** swabs

Chick number	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14
1	---/-	---/-	++-/-	+++/-			+++/-	/+	+++/-	+a+/-	-++/-	---/-		
2	---/-	-+-/-	+--/-	+++/-	+++/-	+++/+	+++/+	+++/+	+++/-	+++/-	+-+/-	----	---/-	---/-
3	---/-	++a/-	+++/-		†	†	†	†	†	†	†	†	†	†
4	---/-	+++/-	-++/+	+++/+	a++/+	-a+/+	+*+/+	+-+/-	+++/+	++-/-	---/-	---/-		
5	---/-	---/-	+++/-				+*+/+	/+	+++/-	---/-		---/-		
6	---/-	---/-	+++/-				+++/-	/-	+++/-	-+-/-	---/-	---/-		
7	---/-	+++/-	+++/-		†	†	†	†	†	†	†	†	†	†
8	---/-	-+-/-	+++/+				+++/-	/+	+++/-	---/-		---/-		
9	---/-	a-+/-	--+/-	+++/+	†	†	†	†	†	†	†	†	†	†
10	---/-	---/-	+++/-				+++/-	/+	+++/+	---/-		---/-		
11	---/-		nd/-	---/-			nd/-	---/-						
12	---/-		nd/-	---/-			nd/-	---/-						
13	---/-		nd/-	---/-			nd/-	---/-						
14	nd		nd/-	---/-			nd/-	---/-						
15	---/-		nd/-	---/-			nd/-	---/-						
Litrer isolator 3	---/-	+++/-	+-+/-	+++/+	+++/+	+++/+	+--/-	+++/+	---	---/-				
Litter isolator4									---	nd/-				

Notation: egg 1, egg 2, egg 3 / PCR resultaat

*: Allantoic fluid not suitable for HA test.
nd: Not done
a: non-specific sample (embryo died within 24 uur), not tested.
(black) PCR positive, virus isolation negative
(dark grey) Both PCR and VI positive
(light grey) Virus isolation positive PCR negative

DIRECTORY OF NATIONAL LABORATORIES

According to Annex IV of Council Directive 92/40/EEC
(updated 19th July 2002)

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