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

HELD AT:

AVIAN VIROLOGY AND AGROCHEMICAL RESEARCH CENTRE, UCCLE, BRUSSELS, BELGIUM

16th to 18th October 2006

Edited by Dennis J. Alexander

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JOINT TWELFTH ANNUAL MEETINGS OF THE NATIONAL LABORATORIES FOR NEWCASTLE DISEASE AND AVIAN INFLUENZA OF EUROPEAN UNION MEMBER STATES 2006

Programme for Monday 16 October 2006

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

14:00-14:15	**Welcome**	
14:15-14:35	Country Reports on AI based on questionnaires	D. Alexander
14:35-15:00	Update from Russia	V. Irza
	Original contributions on AI	
15:00-15:20	Preventive vaccination in France during winter/spring 2006	V. Jestin
15:20-15:40	Respiratory shedding of H5N1 HPAI in wild ducks infected experimentally	R. Fouchier
15:40-16:00	Tea/Coffee	
16:00-16:20	Influenza pathogenesis studies using mouse models	I. Capua
16:20-16:50	HPAI H5N1 infection of a Mute Swan flock in the city of Torun in Poland	K. Smietanka
16:50-17:10	Sublethal infection of poultry with H5N1 HPAI	I. Brown
17:10-17:30	Review of current status of zoo vaccination in the EU	M. Pittman
17:30-17:50	Vaccination of Swedish zoo birds against avian influenza H5	G. Czifra
17:50-18:10	Title to be confirmed (Data from Hungary)	A. Balint
	Close	

Programme for Tuesday 17th October 2006

09:15-09:35	Surveys for AI in Poultry and Wild Birds	A. Cook
09:35-09:50	Spread of Asian-lineage H5N1 HPAI to Europe	I. Brown

09:50-10:25	Epidemiological analysis of AI Surveillance data and related IT issues	A. Cook T. Stacy
10:25-10:45	**Coffee/Tea**	
10:45-11:05	Human influenza – activities of EISS	A Meijer
11:05-11:15	Flu-Lab-Net	I.Brown
11.15-11.35	Highly pathogenic AI H5N1 in swans, Evros Delta, Greece	T. van den Berg
11:35-11:55	Detection of H6N5 in a turkey flock	G.Koch
11:55-12:15	Recent H3N1 isolation from sentinel mallards	V.Jestin
12:15-13:30	**Lunch**	
13.30-13:50	New molecular methods for pathotyping avian influenza viruses of H5 subtype	T. Harder
13:50-14.10	Antigenic characterisation of H5 viruses by cartography	R.Fouchier
14.10-14.30	Development of N1 ELISA	G.Koch
14.30-14.50	Evaluation of several ELISA kits for Avian Influenza surveillance	T. van den Berg
14.50-15.05	Viral RNA viability in different sample types/processing conditions	M.Slomka
15.05-15.20	Comparison of different methods for the detection of influenza A virus from tracheal and cloacal swabs of chickens infected experimentally	K. Smietanka
15.20-15.35	Tea/Coffee	
15.35-15.55	Ring trial of molecular detection/characterisation methods for AI	M.Slomka
15.55-17.45	Round table discussion of laboratory technical issues to include proposed revisions to the AI diagnostic manual	

Close

Annual meeting of the National Laboratories for Newcastle Disease

Programme for Wednesday 18th October 2006

09:30-09:50	Country Reports on ND based on questionnaires	D. Alexander
09:50-10:05	Technical report from the EU reference laboratory for 2005	I. Brown
10:05-10:30	Report from the European Commission	R. Freigofas M. Pittman
10:30-11:00	**Coffee/Tea**	
	Original contributions on ND	
11:00-11:20	Simultaneous detection and pathotyping of PMV1 by real time RT-PCR	I. Brown
11:20-11:40	Phylogenetic analysis of A/H5N1 HPAI viruses and APMV-1 isolated in Africa in 2006	G. Cattoli
11:40-12:00	Interlaboratory comparative tests	R. Manvell
12:00-12:20	Work programme for Community Reference Laboratory for 2007	M. Pittman
12:20-13:30	Lunch	
13.30-14:30	Discussion, laboratory matters, recommendations etc and close **Close**	

Annual Meeting of the National Laboratories for Avian Influenza

COUNTRY REPORTS ON AVIAN INFLUENZA FOR 2005 BASED ON RESPONSES TO THE QUESTIONNAIRE

Dennis J. Alexander, Ian Brown and Ruth J. Manvell

Community Reference Laboratory for Avian Influenza
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INTRODUCTION

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

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AVIAN INFLUENZA

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

Example response:

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

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

Example response:

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

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

Example response:

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

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

***

RESULTS

A total of 34 questionnaires was sent to different laboratories in 31 countries. Responses were received from 22/25 EU countries [24/27 laboratories]: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece x 2, Hungary, Ireland, Italy, Lithuania, Luxembourg, Netherlands, Poland, Slovakia, Slovenia, Spain, Sweden, UK Great Britain, UK Northern Ireland and from 4/6 [4/7 laboratories] non-EU countries: Bulgaria, Norway, Romania and Switzerland. The samples tested and the results for avian influenza are summarised in the following pages.

VIRUS ISOLATION REPORTS BY COUNTRY

AUSTRIA

Samples tested
None

BELGIUM

Samples tested by inoculation into eggs

Type of bird	Sample	Number
Poultry (chickens and turkey)	Tissue	360
	Cloacal swabs	0
Poultry (others)	Tissue	14
	Cloacal swabs	48
Psittacine	Tissue	10
	Cloacal swabs	30
Duck and geese	Tissue	81
	Cloacal swabs	406
pigeon	Tissue	5
	Cloacal swabs	1
Pet birds	Tissue	6
Quarantine birds	Tissue	199
	Cloacal swabs	307
Ostrich	Tissue	4
Wild birds	Tissue	63
	Cloacal swabs	793

Influenza virus isolated
Wild duck: 1x H3N6

RRT-PCR

Seven samples were examined by RRT-PCR for influenza virus RNA.

BULGARIA

Samples tested by inoculation into eggs

Cloacal swabs, carcasses, internal organs - in eggs

Chicken	129
Domestic waterfowl	126
Turkey	12
Partridge	4
Quail	3
Rock Dove	88
Raven	1
Blackbird	1
Starling	1
Crow	2
Titmouse	1
Stork	3
Wild waterfowl	124
Thrush	1
Great crested grebe	1
Mute Swan	1
Black Coot	4
Golden eye	1
Snipe	2
Corncrake	1
King fisher	3
Mallard	6

Influenza viruses isolated
None.

CYPRUS

Samples tested by inoculation into eggs

Type of bird	Sample	Number
Chicken broilers	tissue cloacal swabs	51 10
Chicken layers	tissue	8
Turkeys	tissue cloacal swabs	7 1
Companion birds	tissue cloacal swabs	5 17
Ostriches	tissue cloacal swabs	11 44
Partridges	tissue cloacal swabs	36 1

waterfowl	tissue cloacal swabs	170 32
Pigeons	tissue	60
Wild birds	tissue cloacal swabs	222 32
Others	tissue cloacal swabs	17 52

Influenza viruses isolated
None.

CZECH REPUBLIC

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
layers	tissues	17
broilers	tissues	8
turkeys	tissues	5
pheasants	tissues	3
pigeons	tissues	37
waterfowl	tissues	42
raptors	tissues	4
quarantine birds	faeces	205
other birds	tissues	32

Influenza viruses isolated
None

DENMARK

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens and hens	tissue	320
psittacine and other caged birds	tissue faeces	870 250
ducks and geese	tissue	33
game birds	tissue	70
turkeys and ostriches	tissue	210
pigeons	tissue	14
wild birds	faeces	700*

*in pools of 5

Samples tested by RT-PCR

Wild birds: 2795 faecal samples

Influenza virus isolated

Wild birds 16 AI viruses isolated -see table
140 of the samples tested were positive by RT-PCR for influenza A 27 of these positive in PCR for H5 none for H7. No viruses were isolated from these samples.

Characterisation of AIV isolates

Bird	subtype	IVPI	HA0 cleavage site	conclusion
feral ducks	3 x H3N2			
feral ducks	H5N3		PQRETR*GLF	LPAI H5
feral ducks	H7N5		PEIPKGR*GLF	LPAI H7
feral ducks	H7N7		PEIPKGR*GLF	LPAI H7
feral ducks	2 x H1N1			
feral ducks	H1N3			
feral ducks	3 x H4N6			
feral ducks	H9N1			
feral ducks	2 x H11N9			
feral ducks	H?N2			

ESTONIA

Samples tested by inoculation into eggs:

Cloacal swabs		
Bird species	**Number of birds**	**Pooled samples**
Wild goose	9	4
Crow	1	1
Gull	5	3
Other wild birds	13	7
"Chicken"	18	3
Pewit	1	1
Scolopax	1	1
Bonasa	1	1
Branta	14	5
Swan	1	1
Wood cock	1	1
Wild duck	85	31
Turdus	1	1
Bombycilla (Wax Wing)	5	1
Goldeneye (Bucephala)	3	1

Common Snipe	1	1
Tringa (Yellowlegs)	1	1
Stork	1	1
Pigeon	10	7

Tissue samples		
species	**Number of birds**	**Pooled samples**
Crow	1	1
Domestic goose	1	1
Chickens	14	5
Scolopax	2	2
Finch	1	1
Other wild birds	1	1
Wild duck	3	3
Jay	1	1
Grouse	1	1
Thrush	1	1
Tit	1	1
Pigeons	8	7

Influenza viruses isolated
None

FINLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	tissue samples	32
turkeys	tissue samples	3
geese	tissue samples	10
ducks	tissue samples	7
pheasants	tissue samples	5
cage birds	tissue samples	26
wild birds	tissue samples/cloacal swabs	392

Influenza viruses isolated/detected

	RT-PCR	Virus isolation	subtype	IVPI	no of birds in sample	species
li 9875	positive	positive	H13N6	0.00	3	*Larus argentatus*
li 10202	positive	positive	not H5 or H7		3	*Larus ribibundus*
li 10739	positive	positive	not H5		3	*Larus argentatus*
li 13022	positive	positive	not H5 or H7		5	*Larus argentatus*

FRANCE

Samples tested by inoculation into eggs:

Type of bird	Sample	Number	mRRT-PCR
free range chickens	pooled tissues	5	
chickens/layers	cloacal swabs	10	
	tracheal swabs	4	
	pooled tissues	112	
ducks breeders	cloacal swabs	62	
	pooled tissues	3	
meat pigeons	pooled tissues	2	
turkey breeders	cloacal swabs	176	5
	tracheal swabs	112	
	pooled tissues	5	
meat turkeys	pooled tissues	4	3
pheasants	tracheal swabs	5	
	pooled tissues	5	
Guinea fowl	pooled tissues	4	
force fed ducks	cloacal swabs	15	960
Ornamental birds	cloacal swabs	5	
	pooled tissues	3	
hobby chickens	cloacal swabs	2	
	tracheal swabs	2	
hobby pigeons	cloacal swabs	11	
	tracheal swabs	5	
	pooled tissues	38	
teals	pooled tissues	2	
wild pigeons	cloacal swabs	6	
	tracheal swabs	6	
	pooled tissues	4	
wood pigeons	pooled tissues	4	
gull	pooled tissues	1	
swans	pools		10
other wild birds	pooled cloacal swabs	2	1077

Influenza viruses isolated

Turkeys breeders: 3 x H1N1, 1 x H1N2
Ducks for force feeding: 1 x H2N3, 1 x H4N1, 1 x H5N1, 2 x H5N2, 1 x H5N3, 1 x H6N1, 4 x H6N2, 2 x H6N8, 1 x H11N9
Wild Birds: Teal 1 x H3N8, 2 x H4N6, 1 x H6N8
Mallard: 1 x H5N? only detected by RT-PCR, not isolated ; negative with PCR N1, N2 and N3

Characterisation of AIV isolates

Bird	Subtype	IVPI	HA0 cleavage site	Conclusion
Turkeys breeders	3 x H1N1	0.0	n/a	LPAI
	1 x H1N2	0.0	n/a	LPAI
Ducks for force feeding	1 x H2N3	0.0	n/a	LPAI
	1 x H4N1	0.5	n/a	LPAI
	1 x H5N1	0.0	PQRETR*GLF	LPAI
	2 x H5N2	0.0	2 cleavage sites determined : 1 PQKETR*GLF 1 PQRETR*GLF	LPAI
	1 x H5N3	0.0	PQRETR*GLF	LPAI
	1 x H6N1	0.0	PQIETR*GLF	LPAI
	4 x H6N2	0.0	same cleavage site : 4 x PQIETR*GLF	LPAI
	2 x H6N8	0.0	same cleavage site : 2 x PQIETR*GLF	LPAI
	1 x H11N9	0.0	n/a	LPAI
Wild Birds	1 x H3N8	0.0	n/a	LPAI
	1 x H4N6	0.3	n/a	LPAI
	1 x H4N6	0.0	n/a	LPAI
	1 x H5N?	no isolate	PQRETR*GLF	LPAI
	1 x H6N8	0.0	n/a	LPAI

GERMANY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	cloacal swabs	8
turkeys	cloacal swabs	3
ducks	cloacal swabs	103
geese	cloacal swabs tissue samples	505 3

RT-PCR
In summary, 9078 wild bird samples were processed by real time RT PCR in 2005 of which 223 were positive for presence of AIV M gene-specific sequences. Among these 25 samples were also positive for H5 gene-specific sequences.

Influenza viruses isolated

meat turkeys 1 x H3N8
ostrich 3 x H5N3

wild waterfowl 1 x H1Nx, 5 x H3N8, 1 x H4Nx, 11 x H4N6, 4 x H5N2, 1 x H6N1, 2 x H6N2, 2 x H6N8, 1 x H9N2, 5 x H10Nx

Characterisation of AIV isolates

Bird	subtype	IVPI	HA0 cleavage site	conclusion
turkeys	H3N8	to be done	nd	LPAI
ostriches	3 x H5N3	n.d.	3 x PQRETR*GLF	3 x LPAI
mallards	4 x H5N2	n.d.	4 x PQRETR*GLF	4 x LPAI

GREECE - THESSALONIKI

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Broilers	cloacal swabs	69
	tissue samples	18
Layers	cloacal swabs	131
	tracheal swabs	98
Meat turkeys	cloacal swabs	16
	tracheal swabs	14
ducks/geese	cloacal swabs	231
	tracheal swabs	51
psittacines	cloacal swabs	125
	tracheal swabs	3
pigeons	cloacal swabs	39
	tissue samples	10
quail	cloacal swabs	145
partridges	tracheal swabs	54
waterfowl	cloacal swabs	365
shore birds	tracheal swabs	331
other wild birds	cloacal swabs	548
	tissue samples	290

Influenza viruses isolated
None

GREECE - ATHENS

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissue samples	120
layers	tissue samples	43
breeders	tissue samples	10
pigeons	cloacal swabs	72

wild birds	cloacal swabs	62
canaries	cloacal swabs	8
pheasants	cloacal swabs	8
quails	cloacal swabs	6
turkeys	cloacal swabs	5

Influenza viruses isolated
None

HUNGARY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
non defined species	tracheal swabs	6
zoo birds	tissue samples	7
collared dove	tissue samples cloacal swab	18 1
teals (incl. *Aythya farina, Anas crecca*,)	cloacal swabs tracheal swabs tissue samples	309 735 17
tit	cloacal swabs	26
mute swan	tracheal swab tissue samples	1 19
seagull	tissue sample cloacal swabs tracheal swabs	3 8 2
crane	tissue samples cloacal swabs	13 98
pet birds	tissue samples	13
Eurasian buzzard	tissue sample tracheal swabs cloacal swabs	1 1 3
pheasant	tissue sample cloacal swabs tracheal swabs	22 2 33
blackbird	tissue sample	1
partridge	tissue sample	6
quail	tissue sample	1
pigeon	tissue samples	118
barn Owl	tissue sample	1
Guinea-fowl	tissue samples	10
duck	tissue sample tracheal swabs	26 15
great cormorant	tissue sample cloacal swabs tracheal swabs	2 9 20
sparrow hawk	tissue samples	3

goose	tissue samples tracheal swabs	7 6
reed bunting	cloacal swabs	2
great white egret	cloacal swabs	5
white fronted goose	cloacal swabs tracheal swabs	93 3
grey lag goose	cloacal swabs	15
wren	cloacal swabs	3
parrot	tissue samples	5
finch	tissue sample	1
turkey	tissue sample	17
oriole	tissue samples	13
tattler	cloacal swabs	7
starling	tissue sample	21
coot	cloacal swabs	7
magpie	tissue sample	1
grey heron	cloacal swabs	13
chicken	tissue sample	70
other wild birds	tissue samples cloacal swabs tracheal swabs	1174 158 65
sparrow	tissue samples	*2*
bean goose	cloacal swabs	10
other wild goose	tissue samples cloacal swabs tracheal swab	9 14 26
carrion crow	tissue samples tracheal swabs	13 40
kestrel	tissue sample	1
robin redbreast	tracheal swab	1

Influenza viruses isolated
None

IRELAND

Samples tested by inoculation into eggs:

Type of bird	**Sample**	**Number**
broilers	tissue samples	12
chickens	tissue samples	64
ducks	tissue samples cloacal swabs tracheal swabs	14 15 15
pigeons	tissue samples	22
exotic birds	tissue samples	19
geese	tissue samples	7
turkeys	tissue samples	6

wild birds	cloacal swabs	486
other	tissue samples	17

Influenza viruses isolated
None

ITALY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	pools of cloacal swabs tissue samples pools of tracheal swabs	41 74 4
layers	pools of cloacal swabs tissue samples	1 3
meat turkeys	pools of tracheal swabs tissue samples	787 1
domestic duck	pools of cloacal swabs pools of tracheal swabs tissue samples pools of faeces	406 339 67 22
ostriches	pools of cloacal swabs pools of faeces	30 5
quail	tissue samples	2
pigeons	tissue samples	2
parrots	pools of faeces	1
geese	pools of cloacal swabs pools of faeces pools of tracheal swabs	25 2 1
Guinea fowl	pools of tracheal swabs tissue samples pools of faeces	1 1 1
mallards	pools of cloacal swabs pools of tissue samples pools of tracheal swabs	107 1 3
other birds	pools of cloacal swabs pools of faeces pools of tracheal swabs	310 23 1

Samples from wild birds:		
mallard *(Anas platyrhynchos)*	cloacal swabs tissue samples	128 5
teal *(Anas crecca)*	cloacal swabs tissue samples	41 2
pintail *(Anas acuta)*	cloacal swabs	1
gadwall *(Anas strepera)*	cloacal swabs	2
shoveller *(Anas clypeata)*	cloacal swabs tissue samples	10 1
coot *(Fulica atra)*	cloacal swabs	1
dunlin *(Calidris alpina)*	cloacal swabs	17
black-headed gull *(Larus ridibunot doneus)*	cloacal swabs	3
Ruff (*Philomachus pugnax*)	cloacal swabs	13
Eurasian wigeon (*Anas Penelope*)	cloacal swabs tissue samples	23 1
Common pochard (*Aythya farina*)	cloacal swabs	1
Sandwich tern (*Sterna sandvicensis*)	cloacal swabs	11
Little ringed plover (*Charadrius dubius*)	cloacal swabs	1
Flamingo (*Phoenicopterus ruber*)	cloacal swabs	19
Mediterranean gull (*Larus melanocephalus*)	cloacal swabs	1
Garganey (*Anas querquedula*)	cloacal swabs	2
Whiskered tern (*Chlidonias hybrida*)	cloacal swabs	1
Wood sandpiper (*Tringa glareola*)	cloacal swabs	10
Squacco heron (*Ardeola ralloides*)	cloacal swabs	1
Common tern (*Sterna hirundo*)	cloacal swabs	4
Starling (*Sturno vulgaris*)	cloacal swabs	1

Influenza viruses isolated

From intensively reared birds
turkeys 3 x H5N2

From backyard flocks
Pekin ducks 1 x H4N6, 1 x H10N6, 1 x H10N7, 1 x H11N2, 2 x H11N9
geese 1 x H2N2
mallards 1 x H4N6, 1 x H10N7, 1 x H11N9

Guinea fowl 1 x H9N2

From wild birds
mallard *(Anas platyrhynchos)* 1 x H1N1, 1 x H3N8, 1 x H4N6, 1 x H5N1, 1 x H7N7, 1 x H9N2, 1 x H10N7, 1 x H10N8, 1 x H11N9
teal 1 x H1N1, 1 x H1N3, 1 x H5N2, 1 x H5N3, 2 x H7N7
wigeon (Anas penelope) 1 x H6N9
shoveler (Ana clypeata) 1 x H10N7
flamingo (Phoenicopterus ruber) 1 x H6N2

Characterisation of AIV isolates

Bird	subtype	IVPI	HA0 cleavage site	conclusion
meat turkey	H5N2	0.0	PQRETR*GLF	LPAI
meat turkey	H5N2	not done	PQRETR*GLF	LPAI
meat turkey	H5N2	not done	PQRETR*GLF	LPAI
teal	H5N2	not done	PQRETR*GLF	LPAI
teal	H5N3	not done	PQRETR*GLF	LPAI
teal	H7N7	not done	PEIPKGR*GLF	LPAI
teal	H7N7	not done	PEIPKGR*GLF	LPAI
wild mallard	H7N7	not done	PEIPKGR*GLF	LPAI

LITHUANIA

No investigations were undertaken.

LUXEMBOURG

Type of bird	Sample	Number
poultry	organs	6
	faeces	3
goose	faeces	3
tawny owl	faeces	1
barn owl	faeces	2
pochard	faeces	6
mallard	faeces	2
teal	faeces	3
common snipe	faeces	3
water rail	faeces	1
woodcock	faeces	1
	organs	1
pigeon	organs	1
swan	faeces	6
	organs	1
gull	faeces	5
rook	organs	1
unknown	organs	1
unknown	faeces	4
coot	faeces	5
duck	faeces	2
moorhen	faeces	2

Influenza viruses isolated
None

NETHERLANDS

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	trachea swabs	65
	trachea	55
	lung	55
turkeys	trachea	5
	lungs	5
coots	trachea	5
	lung	5
gulls	trachea	5
	lung	5
psittacines	swabs	2
poultry	trachea	14
	cloaca	14
	swabs	14
geese	trachea	14
	lung	14
ducks	trachea	20
	lung	20
	cloaca swabs	40
sparrows	trachea	4
	lungs	4
swans	lung	22
	trachea	22
birds	lung	25
	trachea	25
	unspecified	6
waterbirds	cloaca swabs	78
	trachea swabs	78
wild birds:		
ducks	swabs	28
coots	swab	1
swans	swabs	2
divers	swabs	7
Exotic birds (Q:)		
parrots	cloaca swabs	360
	faeces	12
	manure samples	36
birds	cloaca swab	602
	manure samples	76
	faeces samples	30
pigeon	manure	1

Influenza viruses isolated
None

NORWAY

Samples tested

Bird	Specimen	Detection method	Result
wild duck	419 cloacal swabs	RT-PCR + inoc. in eggs of positive RT-PCR samples	80 RT-PCR positive (62 subtyped:H1, H2, H3, LPAI H5N1, LPAI-H5N2, H6, H8, H9, H11, H12 and 18 untyped but H5/H7 negative) 10 AI isolation positive
wild goose	194 cloacal swabs	RT-PCR	Negative

Influenza viruses isolated

10 AI-isolation positive from wild ducks (wild bird surveillance program):

2 X H3, 1 X LPAI-H5N2, 6 X H6, 1 X H9

Characterisation of AIV isolates

Bird	subtype	IVPI	HA0 cleavage site	Conclusion
mallard*	H5N1	n.d	PQRETR*GLF	LPAI
mallard*	H5N2	n.d	PQRETR*GLF	LPAI
teal*	H5N2	n.d	PQRETR*GLF	LPAI

* AI surveillance programme on wild birds

POLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	tissues	25
pheasants	faeces	40
ducks	tissues	5
pigeons	tissues	10
geese	cloacal swabs	80

Influenza viruses isolated
None

In addition 277 dead wild birds were tested by RT-PCR for the matrix gene with negative results

ROMANIA

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	tissue sample	107
	cloacal swabs	391
turkeys	tissue sample	25
	cloacal swabs	81
swans	tissue sample	3
	cloacal swabs	12
grey heron	cloacal swabs	10
wild geese	tissue sample	2
	cloacal swabs	2
ducks	tissue sample	4
	cloacal swabs	152
moorhen	tissue sample	1
	cloacal swabs	1

Influenza viruses isolated

35 isolates were obtained all were H5N1 from :

chickens 22 x H5N1
turkeys 3 x H5N1
Swans 5 x H5N1
grey heron 1 x H5N1
wild goose 1 x H5N1
ducks 2 x H5N1
moorhen 1 x H5N1

Characterisation of AIV isolates

Bird	subtype	IVPI	HA0 cleavage site	conclusion
chickens	H5N1		PQGERRRKKRGLF	HPAI
turkeys	H5N1		PQGERRRKKRGLF	HPAI
swans	H5N1	2.85	PQGERRRKKRGLF	HPAI
grey heron	H5N1		PQGERRRKKRGLF	HPAI
wild goose	H5N1		PQGERRRKKRGLF	HPAI
ducks	H5N1		PQGERRRKKRGLF	HPAI
moorhen	H5N1		PQGERRRKKRGLF	HPAI

Molecular analyses were performed by Veterinary Laboratories Agency-Weybridge or by IDAH, and the IVPI by Veterinary Laboratories Agency-Weybridge.

SLOVAKIA

Virus isolation attempts:

Type of bird	Samples	Number	Method
broilers	tissue samples	6	in cell culture
	tissue samples	20	in eggs
	cloacal swabs	5	in eggs
layers	tissue samples	17	in cell culture
	tissue samples	36	in eggs
	cloacal swab	1	in eggs
	cloacal swab	1	in cell culture
turkeys	cloacal swabs	15	in eggs
pigeons	tissue samples	3	in eggs
	cloacal swab	2	in eggs
	cloacal swab	3	in cell culture
	tissue samples	1	in cell culture
wild birds	tissue samples	13	in eggs
	cloacal swabs	5	in cell culture
	cloacal swabs	33	in eggs
	blood	1	in eggs
other birds	cloacal swabs	7	in cell culture
	cloacal swabs	31	in eggs
	tissue sample	6	in eggs
	faeces	12	in eggs

Influenza viruses isolated
None

SLOVENIA

Samples tested by inoculation into eggs and by PCR:

Type of bird	Sample	Number in eggs	Number by PCR
broilers	cloacal swabs	30	30
layers	cloacal swabs	16	
	tracheal swabs	8	
turkeys	cloacal swabs	16	17
	tissue sample	1	
galliformes	cloacal swabs	8	8
	tissue sample	1	
pigeons	cloacal swabs	15	15
	tissue samples	2	2
ducks and geese	cloacal swabs	51	47

	tissue samples	2	
herons	cloacal swabs	10	10
	tissue samples	4	4
Charadriiformes	cloacal swabs	5	5
Gruiformes	cloacal swabs	1	1
Pelecaniformes	cloacal swabs	7	7
Podicepediformes	cloacal swabs	2	2
Psitacidae	cloacal swabs	5	5
Passeriformes	cloacal swabs	20	20
others	cloacal swabs	7	7

Influenza viruses isolated
None

SPAIN

Samples tested by inoculation into eggs

Type of bird	Sample	Number
blackbird (mirlo)	cloacal swabs	6
broiler	cloacal swabs	25
canary	cloacal swabs	390
chicken	cloacal swabs	45
collared dove	cloacal swabs	13
duck	cloacal swabs	151
falcon	cloacal swabs	21
Griffon vulture	cloacal swabs	13
gull	cloacal swabs	2
golden oriole	cloacal swabs	2
ostrich	cloacal swabs	10
pigeon	cloacal swabs	26
partridge	cloacal swabs	11
psittacines, exotic/pet/quarantine	cloacal swabs	2813
quail	cloacal swabs	2
starling	cloacal swabs	16
sparrow hawk	cloacal swabs	3

Influenza viruses isolated

Bird	subtype	IVPI	conclusion
duck	H6N2	0.00	LPAI
wild bird	H2N9	nd	LPAI

SWEDEN

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
imported layer breeders	tissue samples	4
layers	tissue samples	12
backyard poultry	tissue samples	4
wild birds (mallards)	tissue samples	4
zoo birds	tissue samples	1

Influenza viruses isolated
Wild birds (mallards) 1 x H5N3

Bird	subtype	IVPI	HA0 cleavage site	conclusion
mallards	H5N3	nd	PQRETR*GLF	LPAI

SWITZERLAND.

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
broilers	tissue samples	1
laying hens	tissue samples	5
fancy breed	tissue samples	1
pet birds	tissue samples	1
pigeons	tissue samples	1

Influenza viruses isolated
None

UK GREAT BRITAIN

Samples tested

Type of bird	Sample	Method	Number
chickens	tissues	eggs	300
		cell culture	204
	cloacal swabs	eggs	247
		cell culture	247
turkeys	tissues	eggs	13
		cell culture	43
	cloacal swabs	cell culture	41
caged birds	cloacal swabs	eggs	325
		cell culture	38
pheasants	tissues	eggs	58
		cell culture	15
	cloacal swabs	eggs	131
		cell culture	56
pigeons/doves	tissues	eggs	100

	cloacal swabs	cell culture eggs	100 120
waterfowl	cloacal swabs	eggs cell culture	148 111
raptors	tissues cloacal swabs	eggs cell culture eggs	34 29 20
penguins	tissues	eggs	24
ratites	tissues	eggs	12
other	tissues	eggs cells	20 20

Influenza viruses isolated

waterfowl 1 x H6N2 (mallard)
cage birds in quarantine 6 x H5N1 (messias)

Bird	subtype	IVPI	HA0 cleavage site	conclusion
mallard	H6N2	0.00	nd	LPAI
messias	H5N1	2.88	PPRERRRKRGLF PLRERRRKRGLF x 5	HPAI HPAI

UK NORTHERN IRELAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	tissues	11
turkeys	cloacal swabs tissues	60 3
canaries	tissues	5
African grey parrot	cloacal swabs	2

Influenza viruses isolated
None

DISCUSSION

Responses
The responses for the last 5 years compared to the number of countries invited to complete the questionnaire have been:
2000 19/29; 2001 22/29; 2002 25/30; 2003 28/30, 2004 22/30.
For 2005 31 countries were sent the questionnaire and 26 responded, which corresponded to 28 of 34 laboratories.

Samples tested

The overall isolation attempts for avian influenza are summarised in Tables 1 to 3 for egg inoculations and Table 3 for cell culture inoculations.

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

Bird	Number countries	Number
chickens	24	1736
turkeys	12	321
ducks & geese	13	416
game birds etc	10	234
ostriches	4	24
pigeons	20	656
cage, zoo & Q	11	1394
wild birds	15	2400
others	6	151
TOTAL		**6932**

Table 2 Summary of virus isolation attempts in eggs from cloacal swabs, and faecal samples by countries responding to the questionnaire

Bird	Number countries	Number
chickens	10	2109
turkeys	10	517
ducks & geese	11	2099
game birds etc	9	359
ostriches	3	93
pigeons	10	305
cage, zoo & Q	11	4876
wild birds	21	6556
others	3	75
TOTAL		**16,989**

Table 3 Summary of virus isolation attempts in eggs from oropharyngeal swab samples by countries responding to the questionnaire

Bird	Number countries	Number
chickens	7	239
turkeys	2	803
ducks & geese	6	496
game birds etc	3	92
ostriches	0	0
pigeons	3	18
cage, zoo & Q	3	3
wild birds	5	2061
others	2	7
TOTAL		**3719**

Table 4 Summary of virus isolation attempts from all samples* in cell cultures by countries responding to the questionnaire

Type of bird	Number countries reporting attempts	Number
chickens	2	475
turkeys	1	84
ducks & geese	1	111
game birds	1	61
pigeons	3	244
cage, zoo, pet, quarantine etc	1	38
others	1	29
wild birds	1	5
TOTAL		**1048**

* tissues/tracheal swabs/cloacal swabs/faeces

To some extent the responses in the questionnaires were confused by the surveillance exercises undertaken in the EU during 2005 in that some countries included that data while others did not. Similarly, some countries included PCR data in their returns while other did not.

Viruses isolated

Of the 28 laboratories responding 17 reported no isolations of AI viruses. These were Austria, Bulgaria, Cyprus, Czech Republic, Estonia, Greece x 2, Hungary, Ireland, Lithuania, Luxembourg, Netherlands, Poland, Slovakia, Slovenia, Switzerland, and UK Northern Ireland.

HPAI

HPAI isolates were restricted to 25 outbreaks in poultry and 10 isolates from wild birds in Romania, plus isolates obtained from a single outbreak in mesias (*Leiothrix argentauris*) at a quarantine station in Great Britain. These are summarised in Table 5.

Table 5 Summary of HPAI viruses isolated.

Subtype	Country	Bird	Number	HA0 cleavage site
H5N1	Romania	chickens	22	PQGERRRKKR*GLF
H5N1	Romania	turkeys	3	PQGERRRKKR*GLF
H5N1	Romania	wild birds	10	PQGERRRKKR*GLF
H5N1	UK-GB	mesias-Q	6	PPRERRRKR*GLF PLRERRRKR*GLF x 5

LPAI H5 and H7 subtypes

The H5 and H7 LPAI viruses isolated in the EU during 2005 are summarised in Table 6.

Table 6 Summary of H5 or H7 subtype LPAI viruses isolated.

Subtype	Country	Bird	Number	HA0 cleavage site
H5N1	France	force fed ducks	1	PQRETR*GLF
	Norway	mallard	1	PQRETR*GLF
H5N2	France	force fed ducks	1	PQKETR*GLF
	France	force fed ducks	1	PQRETR*GLF
	Germany	mallards	4	PQRETR*GLF
	Italy	meat turkeys	3	PQRETR*GLF
	Italy	teal	1	PQRETR*GLF
	Norway	teal	1	PQRETR*GLF
	Norway	mallard	1	PQRETR*GLF
H5N3	France	force fed ducks	1	PQRETR*GLF
	Denmark	feral duck	1	PQRETR*GLF
	Germany	ostriches	3	PQRETR*GLF
	Italy	teal	1	PQRETR*GLF
	Sweden	mallard		PQRETR*GLF
H5N?*	France	wild bird	1	PQRETR*GLF
H7N5	Denmark	feral duck	1	PEIPKGR*GLF
H7N7	Denmark	feral duck	1	PEIPKGR*GLF
	Italy	teal	2	PEIPKGR*GLF
	Italy	wild mallard	1	PEIPKGR*GLF

*virus not isolated detected by RTPCR

Other LPAI viruses

A total of 97 LPAI influenza viruses of subtypes other than H5 or H7 was isolated from 9 countries (Table 7). Most of the isolates were from wild waterfowl and reflected the active surveillance carried out during 2005. There were no isolates of these viruses from chickens.

Table 7 Summary of other LPAI viruses isolated by countries responding to the questionnaire

Type of bird	Subtype	No. of isolates	No. Countries
turkeys	H1N1	3	1
	H1N2	1	1
	H3N8	1	1
ducks [poultry]	H4N6	2	1
	H6N1	1	1
	H6N2	5	2
	H6N8	2	1
	H10N6	1	1
	H10N7	2	1
	H11N2	1	1
	H11N9	4	2
commercial geese	H2N2	1	1
Guinea fowl	H9N2	1	1

wild waterfowl	H1N1	4	2
	H1N3	2	2
	H1N?	1	1
	H3N2	3	1
	H3N6	1	1
	H3N8	6	2
	H3N?	2	1
	H4N6	15	3
	H4N?	1	1
	H6N1	1	1
	H6N2	3	2
	H6N8	2	1
	H6N9	1	1
	H6N?	6	1
	H9N1	1	1
	H9N2	2	2
	H9N?	2	1
	H10N7	2	1
	H10N8	1	1
	H10N?	5	1
	H11N9	3	2
	H?N2	1	1
other wild birds	H2N9	1	1
	H3N8	1	1
	H4N6	2	1
	H6N2	1	1
	H6N8	1	1
	H13N2	1	1

AVIAN INFLUENZA IN RUSSIA: CURRENT SITUATION AND CONTROL STRATEGIES

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Presentation available on website:
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

PREVENTIVE VACCINATION IN FRANCE DURING WINTER/SPRING 2006

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Presentation available on website:
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

RESPIRATORY SHEDDING OF H5N1 HPAI IN WILD DUCKS INFECTED EXPERIMENTALLY

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No presentation available

PNEUMO-AND NEUROTROPISM OF AVIAN ORIGIN ITALIAN HIGHLY PATHOGENIC AVIAN INFLUENZA H7N1 ISOLATES IN EXPERIMENTALLY INFECTED MICE

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Abstract

An experimental infection of mice was performed in order to investigate the potential for interspecies transmission in mammals of Italian HPAI viruses of the H7N1 subtype. Three avian origin isolates were selected, two strains obtained from ostrich (one of which contained a PB2-627 Lysine residue) and one from a chicken. Following intranasal infection of mice, clinical signs and mortality were recorded in the experimental groups challenged with the two ostrich isolates, while only weight loss was observed in those receiving the chicken strain. Viruses were recovered to a varying extent from respiratory

and nervous tissues of infected animals. These results suggest that HPAI viruses, other than H5N1 and H7N7, may have zoonotic implications, and support the consensus that AI infections in poultry are to be eradicated rather than contained.

This has been published:
Pneumo- and neurotropism of avian origin Italian highly pathogenic avian influenza H7N1 isolates in experimentally infected mice. Michela Rigoni, Kyoko Shinya, Anna Toffan, Adelaide Milani, Francesca Bettini, Yoshihiro Kawaoka, Giovanni Cattoli and Ilaria Capua *Virology*,*Volume 364, Issue 1*, *20 July 2007*, *pages 28-35*

HPAI H5N1 INFECTION OF A MUTE SWAN FLOCK IN THE CITY OF TORUŃ IN POLAND

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

SUBLETHAL INFECTION OF POULTRY WITH H5N1 HPAI

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REVIEW OF CURRENT STATUS OF VACCINATION OF ZOO BIRDS AGAINST AI IN THE EU

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

VACCINATION OF SWEDISH ZOO BIRDS AGAINST AVIAN INFLUENZA H5

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

HPAI IN WILD AND DOMESTIC WATERFOWL IN HUNGARY

Dr. Ádám Bálint

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

REPORT ON SURVEYS FOR AVIAN INFLUENZA IN POULTRY AND WILD BIRDS 2005

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

SPREAD OF ASIAN-LINEAGE H5N1 HPAI TO EUROPE

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

EPIDEMIOLOGICAL ANALYSIS OF AI SURVEILLANCE DATA AND RELATED IT ISSUES

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Presentation available on website:
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

EUROPEAN INFLUENZA SURVEILLANCE SCHEME (EISS)

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

FLU-LAB-NET

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Presentation available on website:

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HIGHLY PATHOGENIC H5N1 AVIAN INFLUENZA IN DEAD SWANS, EVROS DELTA, GREECE

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Presentation available on website:

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INTRODUCTION OF AN H6N5 INFLUENZA VIRUS IN TURKEYS

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Presentation available on website:

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RECENT H3N1 ISOLATION FROM SENTINEL MALLARDS

Véronique JESTIN & François-Xavier BRIAND

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

NEW DEVELOPMENTS FOR THE PATHOTYPING OF AVIAN INFLUENZA VIRUSES

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Presentation available on website:

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ANTIGENIC CHARACTERISATION OF H5 VIRUSES BY CARTOGRAPHY

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Erasmus MC, Rotterdam, The Netherlands

No presentation available

VALIDATION OF AN N1 ANTIBODY COMPETITIVE ELISA.

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Introduction.

Vaccination does provide protection against avian influenza but not necessarily against infection and subsequent spread of virus. Therefore the vaccinated population should be monitored for virus infection. Currently, serological monitoring is only possible when using vaccines having a neuraminidase type heterologous to circulating field virus. We validated a commercially available N1 antibody competitive ELISA using sera of experimentally infected birds and negative field sera**.**

Methods and Methods

Elisa
An N1 antibody competition ELISA (FLUAcN1) was purchased from ID-Vet. The ELISA was performed according to the instruction of the manufacturer with slight modification as indicated. Results were expressed as ratio of the signal of sample and of negative control expressed as percentage ($OD_{sample}/OD_{neg.\ control}$x100%). A cut-off value of 45% was recommended by the manufacturer.

Sera
75 chickens were infected with LPAI virus A/Ty/Italy/99 H7N1. Blood was collected at 11 (n=75) and 15 (n=74) days after infection. Sera negative for antibodies against AI viruses were obtained from:

- 78 samples of 4-6 week old broilers vaccinated twice in field against ND
- 84 samples of chickens experimentally infected with A/ch/Penn/83 H5N2

Samples from the Dutch AI monitoring programme negative for antibodies against H5 and H7 viruses:

- 68 samples of ducks
- 48 samples of pheasants
- 20 samples of guinea fowl
- 81 samples of turkeys with antibodies against A/Ty/Neth/2006-H6N5
- 50 samples: 11 of hobby chickens and 39 unspecified birds.

Results

The sensitivity was found to be low (43.6%) when using the incubation time and cut-off value recommended by the manufacturer. However, test sensitivity could be increased when incubating overnight instead of 1 hour as

recommended, whereas this had no effect on test specificity. Moreover, when increasing the cut-off value to 70% of the negative control (i.e. 30% inhibition) test sensitivity was increased without effect on test specificity (table 1).

Table 1 Sensitivity and specificity of N1 antibody competitive ELISA using different cut-off values

Cut-off	Sensitivity (%) incubating for		Specificity (%) incubating for	
	1h at 37 C	o/n at RT	1h at 37 C	o/n at RT
45%	43.6	81.9	100	100
50%	59.1	88.6	100	100
60%	82.6	95.3	100	100
70%	93.3	96.6	81	100

In table 2 the sensitivity and specificity and the 95% confidence limits are shown when using 144 sera of H7N1 infected birds and 427 sera that tested negative for antibodies against influenza virus in an NP antibody competitive ELISA and or the haemagglutination inhibition test or contained antibodies against influenza viruses with N types different from N1. Incubation was overnight and a cut-off value of 70% was used.

Table 2. Relative sensitivity and specificity of N1 antibody competitive ELISA at cut-off value of 70%.

		Infected		Total
		Yes	No	
ELISA	+	71	4	148
	-	3	423	428
Total		74	427	576

	%	Lower limit	Upper limit
Sensitivity	96.1	91.78	100
Specificity	99.1	98.15	100
Predictive value +	94.9	89.98	99.77
Predictive value -	99.3	98.50	100

Conclusions

- The N1 antibody competitive ELISA is suited to detect birds infected with influenza H7N1 virus.
- Using a limited number of sera no cross reactivity of antibodies against N2 and N5 was detected. Moreover reference sera directed against other N types were all negative.
- Sensitivity is increased up to 96% without major effect on specificity if
    - Incubation overnight is used in stead of 1 hour
    - Cut-off is raised from 45 to 60 or 70%
- Further validation of the N1 competitive ELISA is needed using sera of birds vaccinated experimentally and in the field.

EVALUATION OF ELISA KITS FOR AVIAN INFLUENZA SEROLOGICAL SURVEILLANCE

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Presentation available on website:
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

EXPERIENCES WITH AI REALTIME PCR SINCE THE H5N1 THREAT IN EUROPE

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

COMPARISON OF DIFFERENT METHODS FOR THE DETECTION OF INFLUENZA A VIRUS FROM TRACHEAL AND CLOACAL SWABS OF CHICKENS INFECTED EXPERIMENTALLY

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Presentation available on website:
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

THE FIRST PAN-EUROPEAN AI PCR PROFICIENCY PANEL (SPRING 2006)

Marek Slomka

Community Reference Laboratory, Avian Virology, Veterinary Laboratories Agency Weybridge, Surrey, UK

Presentation available on website:
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

Annual Meeting of the National Laboratories for Newcastle disease

COUNTRY REPORTS ON NEWCASTLE DISEASE AND OTHER APMV INFECTIONS FOR 2005 BASED ON RESPONSES TO THE QUESTIONNAIRE

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INTRODUCTION

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

***

NEWCASTLE DISEASE

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

Example response:

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

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

Example response:

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

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

Example response:

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

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

Example response:

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

RESULTS

A total of 34 questionnaires was sent to different laboratories in 31 countries. Responses were received from 22/25 EU countries [24/27 laboratories]: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece x 2, Hungary, Ireland, Italy, Lithuania, Luxembourg, Netherlands, Poland, Slovakia, Slovenia, Spain, Sweden, UK Great Britain, UK Northern Ireland and from 4/6 [4/7 laboratories] non-EU countries: Bulgaria, Norway, Romania and Switzerland. The responses for number of samples processed for ND [APMV-1] are identical to those for avian influenza virus isolations [see above] unless stated below, the results in terms of avian paramyxovirus isolates are summarised in the following pages.

***

VIRUS ISOLATIONS REPORTS BY COUNTRY

AUSTRIA

Samples tested
None

BELGIUM

APMV isolates
pigeons 6 x PPMV1
chickens 6 x APMV-1 (La Sota)
pheasants 1 x lentogenic APMV-1 (Ulster type)
turkey 1 x APMV-1 (La Sota)
other bird 1 x APMV-1 (La Sota)
wild birds 2 x APMV-1

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	mAb group	conclusion
pigeon	ND	RRQKR*F	P	PPMV1
wild birds 1	ND	RQGR*L	?	lentogenic
wild birds 2	ND	RQGR*L	?	lentogenic

BULGARIA

APMV isolates
pigeon 1 x APMV-1
quail 1 x APMV-1
chickens 1 x APMV-1

not characterised

CYPRUS

APMV isolates
Forty one APMV-1 viruses were isolated as follows:

broilers	1
companion birds (canaries parrots)	8
flamingos	3
partridges	2
pigeons	13
wild birds	10
layers chickens	2
fattening turkeys	1
faeces (waterfowl?)	1

Characterisation of APMV-1 isolates

The virus isolated from faeces collected at a dam in November 2005 (waterfowl?) had an ICPI of 1.50 and amino sequence KRKKR*F. The sample was collected in the framework of the surveillance programme for AI.
Twenty one of the 41 isolates were sent to the CRL and identified as vaccine strain Hitchner B1. The high incidence of isolation was due to the vaccination of all backyard flocks and companion birds carried out across the island in January 2005 following the isolation of a virulent strain from two pigeons and a wild partridge late in 2004. The majority of the isolations were made during the three months following the vaccination.

CZECH REPUBLIC

APMV isolates
pigeon 1 x APMV-1

Characterisation of APMV-1 isolates

Bird	ICPI	amino acids	mAb group	genotype	conclusion
pigeon	0.15	^{112}RRQKRF^{117}	nd	nd	PPMV-1

DENMARK

APMV isolates
poultry 1 x APMV-1 from broiler breeders
wild birds 3 x APMV1
2 x APMV-4
1 x APMV-6
caged birds in quarantine
1 x APMV-1 from finches
1 x APMV-1 from a cockatoo
5 x APMV-2 from finches
1 x APMV-3 from finches

Characterisation of APMV-1 isolates

bird	ICPI	amino acids 109-124	genotype or mAb group[1]	conclusion
finches	1.88	SGGRRQRR*FIGAVIGS	5b	ND
cockatoo	1.99	SGGRRQKR*FIGAVIGS	5c	ND
fowl[2]	1.79	SGGRRQRR*FIGAVIGS	5b	ND
mallard (wild)	ND	SGGEKQGR*LIGAIIGG	C2/G-Q	lentogenic
mallard (wild)	ND	SGGERQER*LVGAIIGG	H	lentogenic
mallard (wild)	ND	SGGGKQGR*LIGAIIGG	C2/G-Q	lentogenic

[1]Deduced from sequences of the F gene (cleavage site region)
[2]40,00 broiler parent stock

ESTONIA

APMV isolates
Feral ducks 3 x APMV-6

FINLAND

No isolates

FRANCE

APMV isolates
pigeons hobby 7 x PPMV-1
meat pigeons 1 x PPMV-1
ducks [force feeding] 3 x APMV-1 avirulent
pheasant 1 x PPMV-1
pheasant 1 x APMV-1 [detected not isolated].
ornamental birds 1 x APMV-3
chicken (quarantine sentinel) 1 x APMV-3
wild birds 2 x APMV-4

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	genotype	conclusion
pigeon/hobby	1 x 1.1	6 x RRQKRFIG	4b	PPMV-1 virulent
	3 x 1.2			
	1 x 1.3			
	1 x 1.4			
	1 x 1.0	RRRKRFIG		
meat pigeon	1.0	RRQKRFIG	4b	PPMV-1 virulent
pheasant	1.6	RRQKRFIG	4b	PPMV-1 virulent
pheasant	(no isolate)[1]	RRQRRFIG	5b	ND virulent
duck [force feeding]	2 x 0.0	2 x GKQGRLIG	1a	APMV-1 avirulent
duck [force feeding]	< 0.4[2]	GKQGRLIG	1a	APMV-1 avirulent

[1]detected but not isolated; [2]sample had bacterial contamination

GERMANY

APMV isolates

pigeons	53 x APMV-1 (PPMV-1)
	1 x APMV-1 (La Sota)
	2 x APMV-3
layers	2 x APMV-1 (La Sota)
turkeys	2 x APMV-1 (La Sota)
aviary birds	5 x APMV-2
	1 x APMV-3
wild birds	4 x APMV-1 (lentogenic)
	1 x.APMV-6

Typing done by use of monoclonal antibodies. Sequences not determined.

GREECE (Athens)

APMV isolates

broilers from 1 holding in Fokida	1 x APMV-1
broilers from 1 holding in Messina	1 x APMV-1
broilers from 1 holding in Evia	1 x APMV-1
broilers from 1 holding in Thessaloniki	1 x APMV-1

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	genotype	conclusion
broilers	1.85	^{112}RRQKRF^{117}	5d	Newcastle disease
broilers	1.80	^{112}RRQKRF^{117}	5d	Newcastle disease
broilers	1.81	^{112}RRQKRF^{117}	5d	Newcastle disease
broilers	1.90	^{112}RRQKRF^{117}	5d	Newcastle disease

GREECE (Thessalonica)

APMV isolates
broilers 2 x APMV-1

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	genotype	conclusion
broilers	1.625	^{112}RRQKR*F^{117}	5d	Newcastle disease
broilers	1.625	^{112}RRQKR*F^{117}	5d	Newcastle disease

HUNGARY

APMV isolates

chickens 2 x APMV-1
goose 1 x APMV-1

Characterisation of APMV-1 isolates

Bird	ICPI	amino acids	mAb group	genotype	conclusion
chicken	0.2	-	E	2	vaccine
chicken	0.2	-	E	2	vaccine
goose	0.3	-	E	2	vaccine

IRELAND

APMV isolates
pigeons 1 x PPMV-1
swans 3 x APMV-1

Characterisation of APMV-1 isolates

Viruses not characterised

ITALY

APMV isolates

broilers 8 x APMV-1
rural chickens 1 x APMV-1
pigeons 39 x APMV-1 (PPMV-1)
collared doves 14 x APMV-1 (PPMV-1)
ducks 4 x APMV-1
ducks 1 x APMV-6

In wild birds

teal (*Anas crecca*)	1 x APMV-6
mallard (*Anas platyrhynchos*)	1 x APMV- 4
mallard (*Anas platyrhynchos*)	1 x APMV-?
Eurasian wigeon (*Anas penelope*)	1 x APMV-?
flamingo (*Phoenicopterus ruber*)	1 x APMV-7
robin (*Erithacus rubecula*)	1 x APMV-1 (PPMV-1)

Characterisation of APMV-1 isolates

bird	ICPI	amino acids 112-117	mAb group	genotype	conclusion
broilers	0.0-0.3	GKQGR*L	not identifiable	1	8 x vaccine
rural chicken	0.20	GKQGR*L	not identifiable	1	1 x vaccine
pigeon	0.6-1.4	RRQKR*F	P	4b	39 x PPMV-1
dom.ducks	0.0 0.0-0.5	ERQER*L GKQGR*L	not identifiable	6 1	1 x lentogenic 4 x lentogenic
collared dove	0.7-1.4	RRQKR*F	P	4b	14 x PPMV-1
robin	1.20	RRQKR*F	P	4b	1 x PPMV-1

LITHUANIA

No investigations

LUXEMBOURG

No isolates

THE NETHERLANDS

APMV isolates

chickens	1 x APMV-1
ducks	1 x APMV-1
exotic birds (Q)	4 x APMV-2

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	mAb group	genotype	conclusion
chicken	nd	lentogenic[1]	nd	nd	vaccine
duck	0	nd	nd	nd	vaccine

[1]RT-PCR used to differentiated between lento- and meso/velogenic viruses.

NORWAY

APMV isolates

chickens 1 x APMV-1
wild duck 1 x APMV-1

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	genotype	conclusion
chicken	0.0	^{112}GKQGRL^{117}	a	lentogenic APMV-1
wild duck	nd	^{112}GKQGRL^{117}	b	lentogenic APMV-1

a: Closely related to pheasant/Finland, and duck/Denmark (2003)
b: Closely related to wild duck Far-East/Russia (2001)

POLAND

APMV isolates

feral pigeons 2 x PPMV-1
mallards 1 x APMV-3

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	mAb group	genotype	conclusion
feral pigeon	1.05	^{112}RRQKRF^{117}	P	4b	PPMV-1
feral pigeon	1.00	^{112}RRQKRF^{117}	P	4b	PPMV-1

ROMANIA

APMV isolates

chickens, 96 x APMV-1
red-throated diver *(Gavia stelata)* 1 x APMV-1
pigeons 1 x APMV-1 [PPMV-1]
partridge*(Perdix perdix)* 1 x APMV-1

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	mAb group	genotype	conclusion
chickens	1.32-1.9	nd	nd	nd	Newcastle disease
partridge	1.75	nd	nd	nd	Newcastle disease
diver	1.78	nd	nd	nd	virulent APMV-1
pigeon	1.86	nd	nd	nd	PPMV-1

SLOVAKIA

APMV isolates

pheasants	2 x APMV-1 - 1 holding
turtledove	1 x PPMV-1
wild duck	1 x APMV-1
pigeons	18 x PPMV-1
racing pigeons	5 x PPMV-1 - 1 dovecote

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	genotype	conclusion
racing pigeon	nd	^{112}RRKKRF^{117}	4b	PPMV-1 (5x)
pigeons	nd	^{112}RRQKRFI^{117}	4b	PPMV-1 (12x)
pigeons	nd	^{112}RRKKRF^{117}	4b	PPMV-1 (6x)
turtledove	nd	^{112}RRQKRF^{117}	4b	PPMV-1
pheasants	nd	^{112}GRQGRL^{117}	2	vaccine (2x)
wild duck	nd	^{112}GKQGRL^{117}	1	lentogenic APMV-1

SLOVENIA

APMV isolates

pigeons	3 x APMV-1 [PPMV-1]
mallard	1 x APMV-2

Characterisation of APMV-1 isolates
No further characterisation

SPAIN

APMV isolates

pigeon	1 x APMV-1(PPMV-1)
collared-dove	1 x APMV-1(PPMV-1)
wood pigeon	1 x APMV-7

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	mAb group	conclusion
collared dove	nd	^{112}RRQKRF^{117}	P	PPMV-1
pigeon	nd	^{112}RRQKRF^{117}	P	PPMV-1

SWEDEN

APMV isolates
layers 2 x APMV-1

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	mAb group	genotype	conclusion
layers	1.27	^{112}RRQRR*F^{117}	nd	5b	NDV
layers	1. 85	^{112}RRQRR*F^{117}	nd	5b	NDV

SWITZERLAND

APMV isolates

None

UNITED KINGDOM [GREAT BRITAIN]

APMV isolates

pigeons/doves	17 x APMV-1 [PPMV-1]
	3 x APMV-7
chickens	4 x APMV-1 (lineage 1)
caged birds	1 x APMV-1 (parrot – lineage 1)
	7 x APMV-2
	1 x APMV-3 (African grey parrot)
game birds	3 x APMV-1 (lineage 5b)
owls	1 x APMV-1 (spotted eagle owl- lineage 5b)

Characterisation of APMV-1 isolates

bird	ICPI	amino acids	genotype	conclusion
broiler	0.04	GKQGRL	1	vaccine
pheasant	1.26-1.60	^{112}RRQKRF^{117}	5b	3 x Newcastle disease
pigeon	nd	^{112}RRQKRF^{117}	4b	PPMV-1
parrot	0.00	GKQGRL	1	Newcastle disease
owl	1.41	^{112}RRQKRF^{117}	5b	Newcastle disease

UNITED KINGDOM [NORTHERN IRELAND]

APMV isolates

None

DISCUSSION OF RESULTS

Of the 28 laboratories responding, six, Austria, Finland, Lithuania, Luxembourg, Northern Ireland and Switzerland, reported no isolations of APMV viruses from the samples tested.

The other 22 laboratories reported a total of 427 avian paramyxoviruses. Three hundred and seventy nine of these were APMV-1 viruses (Table 1). Seventy five of these APMV-1 viruses were of low virulence representing the isolation of live vaccine viruses or naturally occurring avirulent viruses. Of the 304 virulent APMV-1 isolates 187 were PPMV-1 viruses from pigeons or doves and 96 were isolates from ND outbreaks in Romania. Viruses characterised as the PPMV-1 variant virus were also isolated from meat pigeons and pheasants in France and a robin in Italy. Six countries reported isolates of virulent APMV-1 viruses from poultry. ND was reported in chickens in Denmark (broiler parents), Sweden (layers) Greece and Romania. An outbreak in pheasants in Great Britain appeared to be related to infection of a pheasant flock in France. The virus was not isolated from the French pheasants, but infection was detected by RT-PCR and the nucleotide sequence of the PCR product indicated the same virus was involved. The outbreaks in Denmark, Sweden France and Great Britain were caused by closely related viruses of genotype 5b.. An unrelated virus [genotype 5d] was responsible for the ND outbreaks in Greece. A total of 187 PPMV-1 isolates were obtained from pigeons and collared doves in 12 different countries and this once again emphasises the continued widespread presence of this virus in Europe

Table 1 Summary of APMV virus isolations reported

Type of APMV	Bird	No. countries	No. isolates
virulent APMV-1	pigeons & doves	12	187
	meat pigeons	1	1
	chickens	4	104
	partridge	1	1
	pheasants	2	5
	caged birds in Q	3	3
	red throated diver	1	1
	robin	1	1
	wild bird faeces	1	1
low virulence APMV-1	chickens	8	28
	ducks	2	6
	turkeys	3	4
	pheasants	2	3
	partridges	2	1
	goose	1	1
	wild birds	6	23
	cage birds	2	9
APMV-2	caged birds in Q	3	16
	aviary birds	1	5
	mallard	1	1

APMV-3	caged birds in Q	2	5
	ornamental/aviary	2	2
	mallard	1	1
APMV-4	wild birds	2	5
APMV-6	wild birds	3	5
	dom. duck	1	1
APMV-7	flamingo	1	1
	pigeons & doves	2	4
APMV-?	wild birds	1	2

SEROLOGY FOR APMV-1

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

ESTONIA

Type of poultry	Number of flocks tested	Number of sera examined	Number of flocks positive	Number of sera positive
chickens	14	2052	0	
quail	1	3	0	

FINLAND

Type of poultry	Number of flocks tested	Number of sera examined	Number of flocks positive	Number of sera positive
broilers	77	4494	1[a]	24[a]
turkeys	33	1671	4[b]	92[b]
layers	16	751	0	0
geese	1	80	1[c]	6[c]

[a]non-specific reaction; [b]maternal antibodies in imported birds; [c]no virus isolated

NORWAY

Type of poultry	Number of flocks tested	Number of sera examined	Number of flocks positive	Number of sera positive
chicken*	111	6699	3	109
chicken**	30	904	0	0
turkey*	6	363	0	0
turkey**	10	295	0	0
dom. geese*	1	61	0	0
dom. geese**	3	93	0	0

dom. duck*	1	63	0	0

*Surveillance; **Import

SWITZERLAND

Type of poultry	Number of flocks tested	Number of sera examined	Number of flocks positive	Number of sera positive
layers	52	536	6	21*
broilers	49	514	21	78*
turkeys	3	30	0	0
fancy breeds	2	11	0	0
pigeons		22		13**
pet birds		119		8***

*during quarantine; **vaccination is allowed in pigeons; ***APMV-3 cross

SWEDEN

Type of poultry	Number of flocks tested	Number of sera examined	Number of flocks positive	Number of sera positive
imported broiler breeders in isolation	11	3269		
broiler breeders	86	5040		
imported layer breeders in isolation	7	2100		
layer breeders	17	1020		
layers	8	298	1	30
imported turkey breeders in isolation	6	1582		
turkey breeders	8	480		
backyard poultry	12	415		
birds from zoo		1		

CONCLUSION

As in previous years it can be concluded from the results reported in the returned questionnaires that there was a low prevalence of ND [virulent APMV-1 infections] in European poultry in 2005. However, there are two areas of concern. Most of the outbreaks reported in Northern Europe since 1996 have been the result of infections with closely related viruses of clade

5b. Due to the isolation of similar viruses from wild birds from time to time, there has been some speculation that there may be a reservoir of these viruses in wild birds. Equally the continued presence of ND in the racing and feral pigeon/dove populations in Europe [an epizootic that now spans 24 years] remains a serious cause for concern and a continuing threat for domestic poultry and wild life.

TECHNICAL REPORT FOR THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2005

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I. LEGAL FUNCTIONS AND DUTIES

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

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2005

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:

    a) Determining the intravenous pathogenicity index (IVPI)

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

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

    d) Limited phylogenetic analysis to assist in epidemiological investigations.

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

Table 1: Number of submissions to the CRL by year since 1988.

1988	1989	1990	1991	1992	1993	1994	1995	1996
401	188	113	154	199	294	385	605	284
1997	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**
227	285	357	704	316	333	464	426	387

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**: 60 %*

WORK DONE: The viruses submitted in 2005 were characterised as shown in Table 2.

Table 2: Identification of viruses submitted to the reference laboratory in 2005

Virus identification	Number
Paramyxoviruses	***179***
Influenza A viruses	***128***
H2N?	3
H2N3	2
H3N?	1
H3N2	1
H3N8	1
H4N?	3
H5N1	47
H5N2	6
H5N3	2
H6N2	1
H7N1	1
H7N4	1
H7N7	8
H8N1	2
H8N4	1
H9N2	31
H10N?	2
H10N4	2
H10N7	8
H11N9	2
H13N?	2
H13N6	1
Untyped	52
Reoviruses	5
Not viable	23

In addition to conventional typing of the viruses submitted a total of 67 AI viruses was subjected to nucleotide sequencing and the amino acids at the haemagglutinin cleavage site deduced. Of these 44 had multiple basic amino acids and therefore were HPAI viruses, 23 had amino acid motifs consistent with virus of low pathogenicity. All of the H5N1 viruses received were HPAI.

The presence of basic amino acids at the haemagglutinin cleavage site is now well accepted as demonstrating virus virulence for AI viruses and intravenous pathogenicity index [IVPI] tests to assess the virulence of the submitted viruses were only done at the request of the submitting country. In all 6 IVPI tests were done on request.

Ongoing and timely phylogenetic analyses of H5, H7 and H9 was done. In particular detailed analyses of emerging H5N1 viruses was done and data presented at SCOFCAH and EU working group meetings as required.

Estimated actual resources: 55%

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

***Work Plan**: Maintenance of existing repository will continue. All viruses submitted to the CRL will be added to the repository after characterisation. Most viruses will be maintained in a frozen state, but selected, representative viruses will be freeze dried. Reagents such as polyclonal chicken antisera, and control antigens will be maintained at levels previous demands have indicated to be necessary to enable characterisation of all 16 H and all 9 N subtypes.*

***% Resources**: 6 %*

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

ANTIGENS: 33ml H1 antigen, 2ml of H2 antigen,34ml H3 antigen, 4ml of H4 antigen, 2524ml of H5 antigen,100ml of H6 antigen,1084ml of H7 antigen,1ml of H8 antigen,135ml of H9 antigen,1ml H10 antigen & 5ml of H16 antigen

In addition, 35 x 1ml of H5N1 reference material (Asian-lineage H5N1 HPAI) was provided for use in molecular diagnostic assays.

ANTISERA: 39ml of H1 antiserum, 20ml of H2 antiserum, 50ml of H3 antiserum, 25ml of H4 antiserum, 280ml of H5 antiserum, 55ml of H6 antiserum, 190ml of H7 antiserum, 20ml of H8 antiserum, 95ml of H9 antiserum, 30ml of H10 antiserum, 20ml of H11 antiserum, 27ml of H12 antiserum, 15ml of H13 antiserum, 20ml of H14 antiserum, 20ml of H15 antiserum and 12ml of H16 antiserum.

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

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	350	7	400	6
H7	300	6	350	7

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

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

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

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

***% Resources**: 6 %*

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

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

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

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

***% Resources**: 3 %*

WORK DONE: *Results were received, analysed and an oral presentation made at the Annual Meeting in 2005. A written report will appear in the proceedings.*

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

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

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

***% Resources:** 2 %*

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

Estimated actual % resources**: **1%

6. Supporting by means of information and technical advice National 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:** 3 %*

WORK DONE: Staff of the CRL were consulted on numerous occasions by other National Laboratories representatives of member states and the Commission including participation in missions. In addition CRL staff took part in the following consultation groups and meetings

1) EU mission to Romania on avian influenza (Ruth Manvell)
2) Mission to South Africa at the request of the South African Government (Ian Brown and Ruth Manvell) to provide advice on control and diagnosis of HPAI principally in Ostriches.
3) OIE ad hoc group on avian influenza (Dennis Alexander)
4) Scientific Committee for the OIE/FAO Network of Expertise on Avian Influenza (OFFLU) [Dennis Alexander, Ian Brown)
5) Scientific Collaborators Committee for the OIE/FAO Network of Expertise on Avian Influenza (OFFLU) [Ruth Manvell]
6) OIE (OFFLU) mission to Russia to assess the avian influenza situation in wildlife and the national measures being taken to minimize the risk of international spread (Ian Brown).
7) European Food Safety Authority Working group on Avian influenza (Dennis Alexander)
8) European Food Safety Authority Working group on 'Migratory birds and their possible role in the spread of highly pathogenic avian influenza wild birds' (Ian Brown)
9) European Food Safety Authority Working group on captive birds (Dennis Alexander)
10) Representing OIE at FAO Inception Workshop in India 22nd August-26th August (Ian Brown)
11) EU Commission Working Group on Revision of the Directive for the Control of Avian Influenza [Ian Brown]
12) EU AI survey guideline revision, Brussels, [Ian Brown]
13) EU Avian Influenza Preparedness Planning. Workshop on improving collaboration between animal and human health surveillance networks in the Community. Luxembourg 28 June 2005
14) Ad-hoc advice in relation to AI global situation especially in SE Asia
15) Coordination of collaboration between animal and human influenza surveillance networks in Europe

16) FAO/OIE/WHO meeting on strategies for control of HPAI in Asia, Ho Chi Minh City, Vietnam, 22-25 February, 2005 [Ian Brown]
17) EU Commission Working Group for revision of guidelines for AI surveillance in wild birds in member states
18) APEC Conference on AI in Taiwan 20th June-27th June 2005 [Ian Brown]
19) 2nd European Influenza Conference in Malta 12th September-14th September 2005, invited speaker on EU AI surveillance [Ian Brown]

Estimated actual % resources**: **20%

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

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

***% Resources:** 2 %*

WORK DONE: In collaboration with the Commission's representatives the Annual Meeting was organised and held at the Belgium NRL [Centre d'Etudes et de Recherches Vétérinaires et Agrochimiques, (CERVA), Brussels] in October 2005.

Estimated actual % resources**: **4%

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

***Work Plan**: Receive and collate submissions edit and produce report of 2004 proceedings before 2005 Annual meeting. Receive and collate submissions of 2005 meeting.*

***% Resources**: 3 %*

WORK DONE: Proceedings of the 2004 meeting were produced before the 2005 meeting.

Estimated actual % resources**: **2%

9. Carry out work in relation to the surveys for avian influenza in poultry and wild birds implemented by Member States during 2004/05, revision of guidelines and production of final report.

***Work Plan**: Produce and distribute panel of reagents, supply technical support. Produce final survey report and ensure guidelines are revised and adopted*

% Resources: 8%

WORK DONE: The CRL produced and held an enlarged panel of reagents. All national laboratories were supplied with reagents for the conduct of the survey. Technical support was provided to the programme including direction on application of the survey and verification of results by CRL if required. All results received from member states were analysed and a final survey report produced. This report contained a detailed epidemiological analyses that was used to inform discussions for revision of the guidelines at a specific meeting held in Brussels with representatives of all member states. The revised guidelines were adopted in decision doc SANCO/10137/2005.

Estimated actual % resources: 6%

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

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

% Resources: 2%

WORK DONE: This was done through CRL staff membership of the WHO/FAO/OIE animal-human influenza network and the various consultations indicated in section 6. In particular closer interaction was achieved with European Influenza Surveillance System (EISS) network for human influenza with a formal meeting held in Luxemborg with various representation from the EU commission. In addition, close watch was kept on situations relating to spread of AI viruses from birds to humans and this is reflected in the publication outputs from CRL staff.

Estimated actual % resources: 2%

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

% Resources: 1%

WORK DONE:

RELEVANT PUBLICATIONS IN 2005

1. ALEXANDER, D.J. (2005). Avian influenza. Chapter 2.7.12. Manual for Diagnostic Tests and Vaccines for Terrestrial Animals. OIE : Paris. http://www.oie.int/eng/normes/MMANUAL/A_00037.htm
2. World Health Organisation Global Influenza Program Surveillance Network; VLA authors, I.H.BROWN, J.BANKS & M.SLOMKA (2005). Evolution of H5N1 avian influenza viruses in Asia. Emerging Infectious Diseases 11 (10), 1515-1521
3. ALEXANDER, D.J., CAPUA, I. & BROWN, I.H. (2005). 1. Avian influenza viruses and influenza in humans. Proceedings of the Frontis Workshop on Avian Influenza, Wageningen http://library.wur.nl/frontis/avian_influenza/index.html
4. ALEXANDER, D.J. (2005). 12. Should there be a change in the definition of avian influenza for legislative control and trade purposes? Proceedings of the Frontis Workshop on Avian Influenza, Wageningen http://library.wur.nl/frontis/avian_influenza/index.html
5. ALEXANDER, D.J. & MANVELL, R.J. (2005). Technical Report of the Community Reference Laboratory for avian influenza 2003. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of Countries of the European Union, VLA Weybridge, 2004 pp. 9-15.
6. ALEXANDER, D.J. (2005) Outbreaks of H5 and H7 avian influenza 1994-2004. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of EU Member States, VLA Weybridge, 2004 pp. 66-85.
7. ALEXANDER, D.J. & MANVELL, R.J. (2005). Country Reports on avian influenza based on responses to the questionnaire. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of EU Member States, VLA Weybridge, 2004 pp 93-114.
8. ALEXANDER, D.J. & MANVELL, R.J. (2005). Comparative tests for antigen identification in different National Laboratories 2004. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of EU Member States, VLA Weybridge, 2004 pp 188-192.
9. ALEXANDER, D.J. (2005). Avian influenza. Abstracts of SGM 156th Meeting Heriot-Watt University Edinburgh
10. ALEXANDER, D.J. (2005). Avian influenza viruses and influenza in humans. Abstracts of OIE/FAO International Scientific Conference on Avian Influenza, Paris p22.
11. ALEXANDER, D.J. & MANVELL, R.J. (2005). OIE Reference Laboratory for Highly Pathogenic Avian Influenza, Annual Report for 2004. Annual Reports of OIE Reference Laboratories and Collaborating Centres 2004. OIE, Paris. pp.
12. I.H.BROWN (2005). Active surveillance for avian influenza in European Union member states. Proceedings of 2nd European Influenza Conference, Malta, 11-14 September 2005, p10.

13. S.C. ESSEN, I.H. BROWN, B. LONDT, M. KOYLASS, R. GARDNER. (2005). Viral genetic factors which influence transmission of influenza virus from avian to mammalian hosts. Proceedings of 2nd European Influenza Conference, Malta, 11-14 September 2005
14. I.H.BROWN, J.BANKS, R.J.MANVELL, S.C.ESSEN, M.SLOMKA, B.LONDT & D.J.ALEXANDER (2005). Recent epidemiology and ecology of influenza a viruses in avian species in Europe and the Middle East. Proceedings of OIE/FAO International Conference on Avian Influenza, Paris, 7-8 April 2005. Developments in Biologicals 124, 45-50.
15. I.H.BROWN (2005). Advances in molecular diagnostics for avian influenza. Proceedings of OIE/FAO International Conference on Avian Influenza, Paris, 7-8 April 2005. Developments in Biologicals 124, 93-98.
16. R.J.MANVELL, R. Horner, G. Akol, C.Abolnik, M. Romito, I.H.BROWN. (2005) Isolation of an influenza A virus subtype H5N2 from ostriches in South Africa in 2004. Full paper submitted for Proceedings of 3rd Ratite International Science Symposium, Madrid, Oct 2005
17. I.H.BROWN (2005). Epidemiology of swine influenza in Great Britain and emerging global issues. Pig Journal 56, 145-150.
18. ALEXANDER D.J. Avian influenza Chapter for OIE Manual of Standards for diagnostic tests and vaccines, OIE : Paris
19. Ilaria Capua, DENNIS J. ALEXANDER, Donald M. Broom, Véronique Jestin, Poul H. Jorgensen, Guus Koch, Stefano Marangon, Maurice Pensaert, Bjorn Olsen, Albert D.M.E. Osterhaus, Alejandro Schudel, Marion Wooldridge Animal health and welfare aspects of Avian Influenza Annex to *The EFSA Journal* (2005) 266, 1-21.

Estimated actual % resources**: **1%

TECHNICAL REPORT FOR THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2005

Ian H. Brown

Community Reference Laboratory for Newcastle disease
Veterinary Laboratories Agency 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 2005

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:

   a) Determining the intracerebral pathogenicity index (ICPI)

   b) Determining basic amino acids composition adjacent to the cleavage site of the FO protein in the virus and phylogenetic analysis

   c) Antigenic grouping of viruses

   d) Limited phylogenetic analysis to assist in epidemiological investigations.

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

1988	1989	1990	1991	1992	1993	1994	1995	1996
401	188	113	154	199	294	385	605	284
1997	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**
227	285	357	704	316	333	464	426	387

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

% Resources: *70 %*

WORK DONE: The viruses submitted in 2005 were characterised as shown in Table 2.

Table 2: Identification of viruses submitted to the reference laboratory in 2005

Virus identification	Number
Influenza A viruses	***128***
Paramyxoviruses	***179***
APMV-1 (NDV)	145
APMV-2	7
APMV-3	2
APMV-7	2
Reoviruses	5
Untyped	52
Not viable	23

In addition to identification and when requested by the submitting country, 43 intracerebral pathogenicity index tests were done on the submitted ND viruses to assess their virulence.

In addition to conventional typing of the viruses submitted a total of 83 APMV-1's were subjected to nucleotide sequencing of an area of the fusion protein gene and the amino acids at the signal sequence through the cleavage site was obtained for *in vitro* assessment of virulence and use in phylogenetic studies.

Estimated actual % resources: 69%

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

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

% Resources: *10 %*

WORK DONE: The 179 APMV viruses received were added to the repository. Reagent stocks were maintained, at least at previous levels [Table 3] and during the year the following were supplied:

ANTIGENS 128 x 1.0ml of Newcastle disease (ND) antigen, 18 x 1.0ml of PMV-2 and 25 x 1.0ml of PMV-3 were supplied.

ANTISERA 62 x 0.5ml of ND antiserum, 6 x 0.5ml of PMV-2 antiserum and 15 x 0.5ml of PMV-3 antiserum were supplied.

363ml of APMV-1 (NDV) inactivated antigen, 40ml of APMV-1 antiserum; 50ml of APMV-2 antigen, 20ml of APMV-2 antiserum; 30ml of APMV-3 antigen, 7ml of APMV-4 antigen, 7ml of APMV-6 antigen, 17ml of APMV-6 antiserum; 9ml of APMV-7 antigen, 18ml of APMV-7 antiserum; 17ml of APMV-8 antigen, 17ml of APMV-8 antiserum; 7ml of APMV-9 antigen, 16ml of APMV-9 antiserum. 13ml of NDV mAb 617/161; 16ml of NDV mAb 7D4; 4ml of NDV mAb U85

***Estimated actual % resources**: 8%*

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

Type	Serum		Antigen	
	Quantity[a]	HI titre[b]	Quantity[a]	HA titre[b]
SPF	100	<1		
NDV	150	8	300	7
APMV-3	100	8	100	8

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

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

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

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

***% Resources**: 6 %*

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

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

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

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

***% Resources**: 3 %*

WORK DONE: *Results were received, analysed and an oral presentation made at the Annual Meeting in 2005. A written report will appear in the proceedings.*

Estimated actual % resources**: **3%

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

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

***% Resources:** 2 %*

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

Estimated actual % resources**: **2%

6. Supporting by means of information and technical advice National 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. In addition staff from the CRL took part in the following consultations.

1. European Food Safety Authority Working group on captive birds (Dennis Alexander)
2. Defra ND Expert Committee (Dennis Alexander and Ian Brown)
3. EU mission to Romania on avian influenza with consequent advice for ND (Ruth Manvell)

***Estimated actual % resources**: 5%*

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

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

***% Resources:** 2 %*

WORK DONE: In collaboration with the Commission's representatives the Annual Meeting was organised and held at the Belgium NRL [Centre d'Etudes et de Recherches Vétérinaires et Agrochimiques, (CERVA), Brussels] in October 2005.

Estimated actual % resources**: **2%

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

***Work Plan**: Receive and collate submissions edit and produce report of 2004 proceedings before 2005 Annual meeting. Receive and collate submissions of 2005 meeting.*

***% Resources**: 3 %*

WORK DONE: Proceedings of the 2004 meeting were produced before the 2005 meeting.

Estimated actual % resources**: **3%

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

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

***% Resources:** 2 %*

WORK DONE: The following publications appeared in 2005 relating to the work of CRL for ND

1. ALEXANDER, D.J. & MANVELL, R.J. (2005). Technical Report of the Community Reference Laboratory for Newcastle disease 2002. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of EU Member States, VLA Weybridge, 2004, pp 116-121.

2. ALEXANDER, D.J. & MANVELL, R.J. (2005). Technical Report of the Community Reference Laboratory for avian influenza 2003. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of Countries of the European Union, VLA Weybridge, 2004 pp. 9-15.
3. ALEXANDER, D.J. & MANVELL, R.J. (2005). Country Reports on Newcastle disease based on responses to the questionnaire. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of EU Member States, VLA Weybridge, 2004 pp 122-132.
4. ALEXANDER, D.J. & MANVELL, R.J. (2005). Comparative tests for antigen identification in different National Laboratories 2004. Proceedings of the Joint 10th Annual Meetings of the National Laboratories for Newcastle Disease and Avian Influenza of EU Member States, VLA Weybridge, 2004 pp 188-192.
5. ALEXANDER, D.J. & MANVELL, R.J. (2005). OIE Reference Laboratory for Newcastle Disease, Annual Report for 2004. Annual Reports of OIE Reference Laboratories and Collaborating Centres 2004. OIE, Paris. Pp
6. ALEXANDER, D.J. & MANVELL, R.J. (2005). OIE Reference Laboratory for Newcastle Disease, Annual Report for 2004. Annual Reports of OIE Reference Laboratories and Collaborating Centres 2004. OIE, Paris. pp.
7. ALEXANDER D.J Newcastle Disease and other avian paramyxoviruses. Chapter 30 for Handbook of American Assoclation of Avian Pathologists
8. ALDOUS E. W. (2005) Molecular investigations into the pathogenicity and epidemiology of Newcastle disease virus. Ph.D. thesis, Reading University, UK.

Estimated actual % resources**: **2%

REPORT FROM THE EUROPEAN COMMISSION

Ramunas Freigofas and Maria Pittman

Health and Consumer Protection Directorate General, European Commission, Brussels, Belgium

Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

DEVELOPMENT OF A REAL-TIME RT-PCR FOR PATHOTYPING NEWCASTLE DISEASE VIRUS ISOLATES USING A NOVEL PROBE

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Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm

PHYLOGENETIC ANALYSIS OF NEWCASTLE DISEASE VIRUSES ISOLATED IN WESTERN AFRICA IN EARLY 2006

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Introduction and objectives

Newcastle disease (ND) is a viral infection of birds caused by an avian paramyxovirus serotype 1 (APMV-1). ND is a highly contagious disease and some strains of virus can cause severe disease in susceptible birds. Newcastle disease is present in vast areas worldwide and is considered to be endemic in Africa.

The majority of people in African countries live in rural areas and virtually every family rears poultry providing the cheapest source of animal protein in the form of eggs and meat. For this reason, poultry farming represents an important resource for the livelihood of villagers in the African continent. Throughout Africa, Newcastle disease is reported to be the most relevant cause of mortality in village chickens causing economic losses and reduced availability of proteins.

During early 2006, within the framework of avian influenza surveillance programmes and Technical Cooperation Programmes (TCP) through FAO, ND viruses were identified in samples collected in Niger, Mauritania and Nigeria from poultry with suspected HPAI.

Little is known about the genetic characteristics of the APMV-1 strains that are currently circulating in these countries and on their epidemiological implications. For this reason partial nucleotide sequences of the fusion protein gene of 13 African ND strains were analysed. The phylogenetic relationships between partial sequences of the fusion protein gene of these African ND viruses and gene sequences from different ND viruses available in the GenBank database were evaluated.

Materials and Methods

Viruses and nucleotide sequence analysis

13 Newcastle disease viruses isolated from poultry in African countries in 2006 were used in this study (7 strains from Mauritania, 5 from Niger and 1 from Nigeria). All viruses were grown in 9 to 10 day old embryonated fowls' SPF eggs. Subtype identification of the viruses was determined by standard haemagglutination inhibition test, as described in EU Council Directive 92/66/EEC. Pathogenicity of isolates were tested by the intracerebral pathogenicity index (ICPI) test and by sequencing of the fusion protein cleavage site.

Viral RNA was extracted from the allantoic fluid using the High Pure™ RNA Isolation Kit (Roche). Amplification of partial segment of the fusion protein gene was carried out by one-step RT-PCR. The PCR products were purified with the High Pure™ PCR product purification kit (Roche) and then subjected to electrophoresis in a 2% agarose gel. PCR products were sequenced using an ABI PRISM BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems). The products of the reaction sequence were purified with an Autoseq™ G-50 kit (Amersham) and run on an ABI PRISM 3100 Avant Genetic Analyzer. Phylogenetic analysis was carried out using the Clustal W software in the MEGA 3 programme (2). Genotyping of Newcastle disease viruses was performed as described by Aldous et al., 2003 (1).

Results

The ICPI values and the deduced amino acid sequences of the fusion protein cleavage site showed velogenic nature of all of the African isolates used in this study. Phylogenetic analysis of the partial sequences of the fusion protein gene (gene F) of the African strains showed that they are all only distantly related to the F gene sequences of the APMV-1 available in GenBank (lower than 91%). Moreover the analysis highlighted that these isolates belong to the genetic lineage 5. In particular they are more similar to ND viruses of the sublineage 5b (1). All of the Mauritanian isolates showed very high percentage of homology to each other (100%), and were distantly related to the strains from Niger and Nigeria. Most of the ND strains from Niger were closely related to each other although two strains, APMV-1/ck/Niger/1377-2/06 and APMV-1/ck/Niger/1377-3/06, showed higher homology with the Nigerian strain than with the remaining strains from Niger.

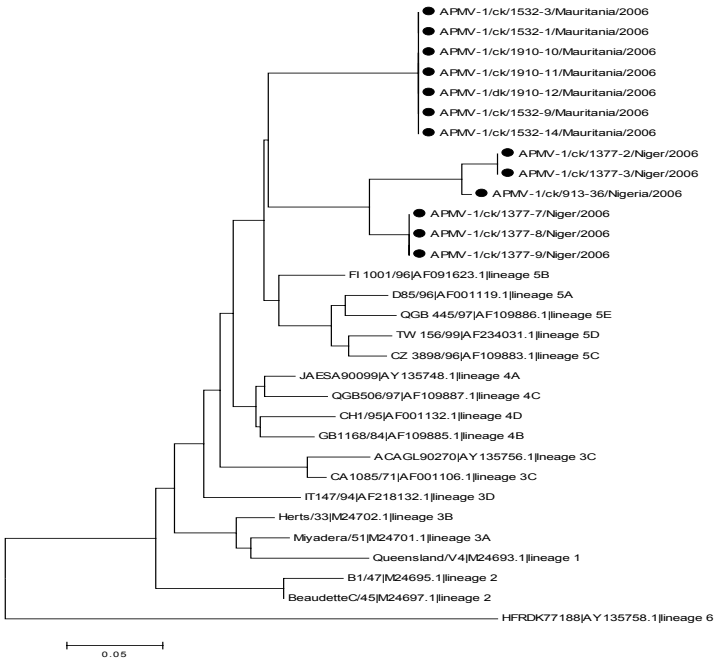
Discussion

By phylogenetic analysis all the African APMV-1 isolates were placed in lineage 5 even if low homology was found between these strains and those published in GenBank. This phylogenetic distance is most probably related to the lack of genetic information on ND viruses currently circulating in Africa. Within lineage 5 the African isolates showed higher homology with strains belonging to sublineage 5b, which is composed also of isolates originating from South Africa. The importance of geographical origin in the evolution of these viruses is highlighted by the high percentage of homology that was found in the gene F sequences of the African strains originating from the same country. In particular this was observed for strains from Niger and for strains from Mauritania. A close relationship was found also between strains originating from neighbouring countries, namely Niger and Nigeria, suggesting transboundary spread. This study confirms that velogenic ND viruses are widely circulating in Niger, Nigeria and Mauritania despite vaccination programmes. Considering the role of rural and scavenging poultry in the livelihood of African villages, more data and investigations are needed to better understand the epidemiology of ND infection in Africa, in order to identify strategic actions to reduce the spread of this viral disease among poultry.

Acknowledgements
The authors wish to thank Dr. L. H. Lombin, Dr. S. Aboubakar, Dr. M. Fall, and their governments for kindly supplying the viruses studied in this analysis.

References
1. Aldous, E. W., Mynn, J.K., Banks, J., Alexander, D. J. (2003). A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *Avian Pathology,32*, 239-257.
2. Kumar, S., K. Tamura and M. Nei. *MEGA3*: Integrated software for molecular Evolutionary Analysis and sequence alignment. Briefings in Bioinformatics. 5 (2): 150-163. 2004.

Phylogenetic tree: the closed circles ● represent viruses used in the present study.



INTER-LABORATORY COMPARATIVE TESTS USING HAEMAGGLUTINATION INHIBITION FOR 2006

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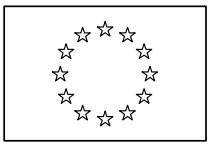
Presentation available on website:

http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/crls_proceedings_en.htm



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/10466/2005
Working document

COMMUNITY REFERENCE LABORATORIES FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE

Work programmes 2006

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2006

I. LEGAL FUNCTIONS AND DUTIES

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

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2006

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:
   a) Determining the intravenous pathogenicity index (IVPI)
   b) Antigenic typing of viruses and both haemagglutinin and neuraminidase subtypes
   c) Determining the amino acid sequence at the haemagglutinin cleavage site of H5 and H7 subtype viruses
   d) Limited phylogenetic analysis to assist in epidemiological investigations.

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

3. Carry out work in relation to the surveys for avian influenza in poultry and wild birds implemented by Member States during 2005/06, revision of guidelines and production of final report.

4. Formally liaise with Public Health Laboratories to ensure rapid flow of information and viruses as appropriate.

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

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

7. To assist MS on the use of PCR techniques and organise the inter-laboratory comparison tests for PCR.

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

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

10. Maintain close awareness of developments in diagnostic methodology and report and advise, as relevant , to Annual Meeting of National Avian Influenza Laboratories.

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

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

13. Establish a genetic database of avian influenza viruses accessible to all national avian influenza laboratories.

14. 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.

15. Provide targeted training in the light of developments for new diagnostic methodology.

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.

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2006

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 2006

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:
   a) Determining the intracerebral pathogenicity index (ICPI)
   b) Determining basic amino acids composition adjacent to the cleavage site of the FO protein in the virus and phylogenetic analysis
   c) Antigenic grouping of viruses
   d) Limited phylogenetic analysis to assist in epidemiological investigations.

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

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

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

5. To assist MS on the use of PCR techniques and organise the inter-laboratory comparison tests for PCR.

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

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

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

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

10. Provide targeted training in the light of developments for new diagnostic methodology.

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

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

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/10662/2006
Working document

COMMUNITY REFERENCE LABORATORIES
FOR
AVIAN INFLUENZA
AND
NEWCASTLE DISEASE
Work programmes 2007

Presented at the 12th Joint Annual Meeting of avian influenza and Newcastle disease laboratories
16-18 October 2006, Uccle, Brussels

WORK PROGRAMME FOR THE
COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2007

I. Legal functions and duties
The functions and duties are specified in Annex VII of Council Directive 2005/94/EC (Official Journal of the European Union of 14.1.2006, No L 10 p.16.)

II. Objectives for the period January – December 2007

1. Characterise viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:

    a. Determining the intravenous pathogenicity index (IVPI)
    b. Antigenic typing of viruses and both haemagglutinin and neuraminidase subtypes
    c. Determining the amino acid sequence at the haemagglutinin cleavage site of H5 and H7 subtype viruses
    d. Limited phylogenetic analysis to assist in epidemiological investigations.

2. Maintain and distribute virus repository and reagents necessary for virus characterisation. Prepare and distribute standard antigens to MS's for use in annual poultry survey.
3. Collate data from surveys for avian influenza in poultry and wild birds implemented by Member States during 2006/7; undertake consistency checks and raise queries with each Member State as required; compile reports on a quarterly and annual basis; undertake epidemiological analyses of these data; recommend improved data collection methods and implement agreed changes

amongst Member States; establish and lead an Epidemiology Working Group to advise on the enhancement of wild bird surveillance and reporting.

4. Review and revise surveillance guidelines for poultry and wild birds as required.
5. Develop and implement IT reporting systems for AI surveillance data subject to approval from MS's.
6. Prepare and distribute antisera, antigens and reagents for the inter-laboratory conventional virological and serological comparison tests.
7. Assist MS on the use of PCR techniques and organise the inter-laboratory comparison tests for molecular detection and characterisation.
8. Analyse results submitted by National Laboratories for the inter-laboratory comparison tests.
9. Conduct work to evaluate reported problem areas in diagnosis.
10. Support by means of information and technical advice National Avian
11. Influenza Laboratories and the European Commission during epidemics.
12. Maintain close awareness of developments in diagnostic methodology and report and advise, as relevant, to the Annual Meeting of National Avian Influenza Laboratories.
13. Prepare the programme and working documents for the Annual Meeting of National Avian Influenza Laboratories.
14. Collect and edit material for a report covering the Annual meeting of National Avian Influenza Laboratories.
15. Ensure genetic data of avian influenza viruses is accessible to all national avian influenza laboratories.
16. Annually review and revise as required the EU AI diagnostic manual.
17. Provide targeted training in the light of developments for new diagnostic methodology.
18. 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.
19. Formally liaise with Public Health Laboratories to ensure rapid flow of information and viruses as appropriate.
20. Preparation and publications of articles and reports associated with above work.
21. It is understood that the above mentioned objectives are not exclusive to other work of more immediate priority which may arise during the given period.

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2007

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 2007

1. Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decisions 95/233/EC and 94/85/EC. This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:

    a) Determining the intracerebral pathogenicity index (ICPI)
    b) Determining basic amino acids composition adjacent to the cleavage site of the FO protein in the virus and phylogenetic analysis
    c) Antigenic grouping of viruses
    d) Limited phylogenetic analysis to assist in epidemiological investigations.

2. Maintain and distribute virus repository and reagents necessary for virus characterisation.
3. Prepare and distribute antisera, antigens and reagents for the inter-laboratory comparison tests.
4. Analyse results submitted by National Laboratories for the inter-laboratory comparison tests.
5. Assist MS on the use of PCR techniques and organise the inter-laboratory comparison tests for PCR.
6. Conduct work to evaluate reported problem areas in diagnosis.
7. Supporting by means of information and technical advice National Newcastle Disease Laboratories and the European Commission during epidemics.
8. Prepare programme and working documents for the Annual Meeting of National Newcastle Disease Laboratories.
9. Collecting and editing of material for a report covering the annual meeting of National Newcastle Disease Laboratories.
10. Provide targeted training in the light of developments for new diagnostic methodology.
11. Preparation and publications of articles and reports associated with above work.

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

COMMUNITY FINANCIAL ASSISTANCE PROVIDED TO CRLS
IN ANIMAL HEALTH AND ZOOTECHNIC 2002 – 2006

CRL	BUDGET 2002	BUDGET 2003	BUDGET 2004	BUDGET 2005	BUDGET 2006
Avian Influenza	150.000	120.000	135.000	135.000	300.000
Newcastle Disease	60.000	60.000	65.000	50.000	70.000
Classical Swine Fever	185.000	190.000	210.000	230.000	220.000
Swine Vesicular Disease	95.000	95.000	95.000	100.000	100.000
African Swine Fever	_____	100.000	105.000	130.000	100.000
African Horse Sickness	40.000	45.000	50.000	35.000	40.000
Fish Diseases	130.000	135.000	140.000	145.000	145.000
Diseases of bivalve molluscs	80.000	85.000	90.000	90.000	90.000
Rabies serology	130.000	130.000	150.000	150.000	165.000
Bluetongue	115.000	120.000	125.000	165.000	200.000
Assessment of bovine breeding	66.000	60.000	65.000	65.000	65.000
EURO TOTAL	1,051.000	1,140.000	1,230.000	1,295.000	1,495.000

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate D – Animal Health and Welfare
D1 - Animal Health and Standing Committees

SANCO/10555/2006 –Rev1

Updated list of names and addresses for AI and ND national reference laboratories in Member States, certain third countries and participants

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National Laboratories for avian influenza and Newcastle disease outside the European Union

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