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

HELD IN BRUSSELS, 9th-10th DECEMBER 1997

Edited by Dennis J. Alexander

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JOINT ANNUAL MEETINGS OF EUROPEAN UNION NATIONAL NEWCASTLE DISEASE AND AVIAN INFLUENZA LABORATORIES 1997

PROGRAMME

Tuesday 9th December 1997

10.45: Welcome and introduction.

11.00: SESSION I **COUNTRY REPORTS FOR 1996-1997**

AVIAN INFLUENZA

1. Belgium/Luxembourg
2. The Netherlands
3. Austria
4. Germany
5. Spain
6. Portugal
7. Greece
8. France
9. Ireland
10. United Kingdom
11. Sweden
12. Finland
13. Denmark
14. Norway
15. Italy

SESSION II **COUNTRY REPORTS - FOR 1996-1997**

NEWCASTLE DISEASE

1. Greece
2. Spain
3. Portugal
4. Italy
5. Austria
6. France
7. Belgium/Luxembourg

8. The Netherlands
9. Germany

12.45: LUNCH

14.00: SESSION II continued

10. Norway
11. Sweden
12. Finland
13. Denmark
14. Ireland
15. United Kingdom
    - Northern Ireland
    - Great Britain

15.50: SHORT BREAK

16.00: SESSION III **EC REPORTS**

16.00: Report from the Commission on Newcastle disease and avian influenza in the European Community. *Jorgen Westergaard*

16.30: Report of the EU Reference Laboratory for avian influenza and Newcastle disease 1996-1997. *Dennis Alexander*

17.00: General discussion of current situation in European Union countries

Wednesday 10th December 1997

10.00: SESSION IV **NEWCASTLE DISEASE IN OSTRICHES**

10.00: Contribution by scientists from South Africa:

- experimental infection of ostriches - virology
- quarantine risk assessment
- control of ND in ostriches in South Africa
- discussion including the risk of importing ostriches and ostrich meat into the EU

11.10: SHORT BREAK

11.20: SESSION V **ORIGINAL CONTRIBUTIONS**

11.20: Acute pancreatitis in chickens due to lentogenic NDV.
Guy Meulemans

11.40: Comparative tests of diagnostic procedures in laboratories in France.
Michele Guittet

12.00: Avian influenza haemagglutination inhibition tests in National Laboratories - Results of the comparison of test reproducibility in different laboratories.
Dennis Alexander

12.45: LUNCH

14.00: SESSION VI **DISCUSSION OF THE FOLLOWING TOPICS**

- current definitions of ND and AI
- capacities for diagnostic testing in National Laboratories
- problems associated with current diagnosis
- problems associated with the HI and AGP tests with ostrich sera
- routine use of PCR for influenza viruses of different subtypes
- use of reference sera and other reagents in National Laboratories
- role of the Community Reference Laboratories in 1998

16.00: **CLOSING REMARKS AND CLOSE**

COUNTRY REPORTS FOR 1996-1997

AVIAN INFLUENZA

The following countries made simple statements of no avian influenza outbreaks during 1996-1997:

Belgium/Luxembourg
The Netherlands
Austria
Spain
Portugal
Greece
Sweden
Finland
Denmark
Norway

AVIAN INFLUENZA - SITUATION IN GERMANY 1995 - 1997

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No outbreak of highly pathogenic avian influenza has occurred in Germany since 1979.

During 1995 to 1997 672 poultry sera from chickens, ducks and turkeys from different flocks for export were screened for subtype H5 and H7 by haemagglutination inhibition tests. No antibodies against H5 and H7 were demonstrated.

During 1995 and 1996 a few influenza virus strains were isolated. Most of them derived from turkey flocks with respiratory symptoms and problems with egg production in breeders (Table 1).

Table 1. Isolation of avian influenza viruses in Germany in 1995/96

Date	Origin	Virus	Subtype†	ICPI	IVPI
08/95	Bav	chicken/Germany/90/95	H9N2	0,00	n.t.¶
09/95	RP	turkey/Germany/106/95	H9N2	0,00	n.t.
09/95	Thur	duck/Germany/113/95	H9N2	0,95	0,00
11/95	L Sax	turkey/Germany/169/95*	H9N2	1,73	1,42
11/95	BW	turkey/Germany/176/95	H9N2	0,06	0,00
01/96	L Sax	turkey/Germany/2/96	H9N2	0,11	n.t.
01/96	L Sax	turkey/Germany/3/96	H9N2	0,00	n.t.
01/96	L Sax	turkey/Germany/4/96	H9N2	0,15	n.t.
01/96	L Sax	turkey/Germany/5/96	H9N2	0,04	n.t.
04/96	L Sax	turkey/Germany/22/96	H9N2	0,00	0,00
05/96	BW	turkey/Germany/33/96	H9N2	0,00	0,00

*mixture of influenza virus H9N2 and velogenic NDV
†the N-Subtype was determined in EU Reference Laboratory Weybridge
¶not tested

All isolates had the antigen subtype H9N2.

The pathogenicity of the isolates for chickens was low and the viruses did not fall within the EU definition of highly pathogenic avian influenza.

One isolate was a mixture of influenza virus subtype H9 and velogenic NDV, and the apparent pathogenicity was high. The virus isolate reacted in the HI test only with influenza antiserum H9 and it was not inhibited by NDV-specific antiserum.

The mixed infection could only be detected by the following methods:
- immunofluorescence test with NDV-specific and influenza-specific conjugated antisera
- polymerase chain reaction
- electron microscopy.

In 1995, 27 turkey flocks in different regions were screened serologically by HI test against subtype H9. Seven of the 12 fattening flocks and 2 of the 15 breeder flocks screened were positive.

In 1996, 4 turkey flocks, and in 1997, 6 flocks were tested, but we could not demonstrate antibodies against subtype H9. However, in one flock we found antibodies against subtype H6.

AVIAN INFLUENZA : SITUATION IN FRANCE FROM OCTOBER 1995 TO OCTOBER 1997

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No cases of avian influenza as it is defined in the E.U. directive 92/40/EEC were reported during the relevant period. Although different egg drop problems occurred in turkey and guinea fowl breeder flocks due to influenza viruses of H6 subtype, the IVPI for these isolates was zero (Table 1).

Other influenza virus infections were suspected but viruses were not isolated. In some circumstances, diagnostic laboratories undertake AGP tests preferentially when birds show clinical respiratory signs, egg drop production, or for import/export controls. In case of doubtful or positive results they send the sera concerned to the national reference laboratory to confirm or not the presence of antibodies by HI tests. In that way, viruses belonging to the subgroups H1, H5, and H6 were suspected to circulate in poultry (Table 2).

Table 1 : Avian influenza viruses isolated

Department	Month Year	Species	Virus subtype	IVPI	Signs
22	Jan. 96	turkey	H6	0	egg drop
49	May 97	turkey	H6	0	egg drop
49	July 97	guinea fowl	H6	0	egg drop
22	Nov. 97	turkey	H6	0	

Table 2 : **Avian Influenza serology (Confirmation of doubtful or positive sera from diagnostic laboratories)**

Department	Month year	Species	AGP	HI	Signs
22	Jan. 96	turkey	+	H1	egg drop
22	Feb. 96	turkey	+	H1	egg drop
44	Jul. 96	emu	-		export
30	Oct. 96	chicken	-		
49	May 96	fowl	-		egg drop
49	Sept. 96	chicken	-		import
44	Jan. 97	ostrich	+	H5	export
22	May 97	turkey	-		egg drop
49	May 97	turkey	+	H6	egg drop
49	May 97	turkey	+	H5	egg drop
49	Jun. 97	guinea fowl	+	H6	egg drop
85	Jul. 97	fowl	-		egg drop
22	Nov. 97	turkey	+	H6	egg drop

REPORT OF THE IRISH NATIONAL REFERENCE LABORATORY FOR NEWCASTLE DISEASE FOR 1996 AND 1997

Gerry Campbell

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1996

There were no outbreaks of avian influenza during 1996

1997

There were no outbreaks of avian influenza falling within the EU definition. An avian influenza virus of H9N2 subtype with an IVPI of 0.00 was isolated from pheasants on a game bird farm.

AVIAN INFLUENZA: SITUATION IN GREAT BRITAIN 1996-1997

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Avian influenza investigations

The incidence of avian influenza (AI) virus infections of turkeys and chickens in Great Britain remained extremely low during 1996-97 (Table 1).

Table 1. Avian influenza investigations in poultry during 1992-1997

	1992	1993	1994	1995	1996	1997
Suspect cases investigated:	2[a]	4	1	2	1	0
Influenza virus isolated:	1[a]	1	1	1	1	0
Confirmed HPAI:	1[a]	0	0	0	0	0

[a]outbreak occurred at the end of December 1991

Isolations of avian influenza viruses from domestic poultry

Only one isolate was obtained from domestic poultry during the period. This was an H4N6 virus with IVPI 0.18 obtained, in September 1996, from a mallard duck showing nervous signs (Table 2).

Influenza isolations from other birds

Although in previous years influenza viruses have been obtained from other birds in Great Britain, especially captive caged birds none were isolated during 1996-97.

Avian influenza viruses from other sources

In 1996 the Oxford Public Health Laboratory submitted a virus isolated from a swab taken from a woman with conjunctivitis. This virus proved to be of H7N7 subtype. The IVPI was 0.00 and the deduced amino acid sequence at the HN0 cleavage site was -PEIPKGRGLF- (1). The woman kept ducks which mingled with feral waterfowl and it was assumed that these were the origin of the virus. It was demonstrated by nucleotide sequencing and phylogenetic comparisons that all eight genes of the virus were closest to viruses of avian origin and that the virus showed close homology with A/turkey/Ireland/PV74/95 (H7N7) (2).

Table 2. Isolations of avian influenza viruses from poultry 1990-1997

Date	County	Virus	Subtype	IVPI
1990				
05/90	Lancashire	duck/England/780/90	H4N6	0.00
1991				
02/91(x2)	Norfolk	duck/England/1194/91	H4N2	0.00
07/91	Norfolk	goose/England/1440/91	H6N8	0.00
12/91	**Norfolk**	**turkey/England/50-92/91**	**H5N1**	**3.00**
1993				
09/93	Norfolk	turkey/England/895/93	H6N8	0.00
1994				
06/94	Norfolk	duck/England/611/94	H4N2	0.00
1995				
10/95	Avon	muscovy/England/1131/95	H4N6	0.15
1996				
		mallard/England/1078/96	H4N6	0.18
1997				
		no isolates		

Isolations of influenza viruses from birds in quarantine.
No viruses were isolated from this source during 1996, but between October to December 1997 nine isolations of H4N6 influenza viruses made from a variety of different species from a single quarantine premises, all had IVPI values <0.7. Viruses of low virulence, usually of H3 or H4 subtypes, have been isolated intermittently from imported captive caged birds since studies were started in 1975 (Table 3).

References

1. Kurtz, J. Manvell, R.J. & Banks, J. (1996). Avian influenza virus isolated from a woman with conjunctivitis. *Lancet* 348, 901902.
2. Banks, J., Speidel, E. and Alexander, D.J. (1998).Characterisation of an avian influenza A virus isolated from a human - is an intermediate host necessary for the emergence of pandemic influenza viruses? *Archives of Virology* **143,** 781-787.

Table 3: Isolations of influenza viruses from birds in quarantine in Great Britain.

DATE	SUBTYPE	NUMBER
1975	H4N6	29
1976-06.1977	H3N8	58
07.1977-1978		NONE
1979	H4N6	2
	H10N7	2
	H7N7	1
1980-06.1987		NONE
1987	H3N8	1
1988	H3N8, H3N6	5
	H4N6	4
1989	H3N8	2
	H4N2, H4N3, H4N6	19
1990	H4N3, H4N8	4
01-06.1991	H4N1, H4N8	4
07.1991-04.1993	NONE	
05 - 08.1993	H4N6	4
08.1993-09.1997	NONE	
10.1997	H4N6	9

THE EPIDEMIOLOGICAL SITUATION OF AVIAN INFLUENZA IN ITALY DURING 1996-1997

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Avian influenza in Italy in 1996

During 1996 three strains of influenza A virus subtype H3N2 of probable avian origin were isolated from pigs and studied. Phylogenetic research is still underway in the Centro Italiano di Referenza per l'Influenza Umana, part of the Istituto Superiore di Sanità in Rome (Drs. I.Donatelli and L.Campitelli), in a joint project with the St.Jude Children's Research Hospital, Memphis, Tennessee, USA (under the direction of Dr. Webster). The three strains have been shown to be of low pathogenicity for chickens (IVPI 0.47). Research is continuing concerning the hypothesis that the genetic reassortment is a mixture of human influenza virus and avian influenza virus which is brought to life in swine which act as a "mixing-vessel".

Yet another strain of influenza virus type A, subtype H9N2, was isolated in 1996 by the Istituto Zooprofilattico in Padua from a pool of chicken organs from the Piemonte region. The farm of origin was a rural type. The strain proved to be of low pathogenicity for chickens by IVPI test.

During 1996 another strain was isolated in a group of 1000 wavy parrots imported from China and under quarantine. The birds were healthy when examined clinically and did not demonstrate anomalous mortality, but cloacal swabs, passed in embryonated eggs, showed the presence of a strain of influenza virus type A, subtype H4N6 with an IVPI of 0 (Istituto Zooprofilattico, Forlì). The presence of this subtype of virus in exotic birds is common and does not provide any evidence of an epidemic.

In addition , during 1996, a joint research project was set up with the Centro Studi Italiano Cetacei which sent fragments of organs from beached cetaceans, in particular parts of lungs and liver from Tursiops, Pilot whale and Zifio, to be analysed. These samples were treated and passed on embryonated eggs, but no haemagglutinating agents have yet been isolated. No outbreaks of avian influenza were found in poultry during 1996.

Avian influenza in Italy in 1997

During 1997 seven strains of avian influenza virus type A were isolated from October in the Istituto Zooprofilattico delle Venezie - Padua Laboratory. All the strains were isolated from rural-type chicken farms, where there was contact with other reared birds, in the Veneto region with the exception of one case in the Friuli Venezia Giulia region (Fig. 1). It is worth emphasising that the north east regions of Italy are crossed by important migratory flyways coming from the east of Europe.

The epidemiological situation has been summarised in Table 1 and it shows that the mortality rate was generally high for chickens, guinea-fowls and turkeys (on average 50%) while there were no clinical signs of influenza in other birds reared on the same farms (ducks, quails, pigeons, pheasants and geese). The strains were isolated over a month and were followed by strict measures of control according to the directive 92/40/EEC which was applied in Italy with DPR 656, 15/11/96.

In particular, each farm was registered as an infected area and all the birds were killed. It is worth noting, (Fig. 2), that it was possible to trace the contact between poultry reared in these farms and wild ducks, and that most of the reared animals were bought from breeders or the live bird market. From a virological point of view (Table 2), the strains were all H5N2 with very high IVPI values and amino acid sequences at the cleavage site of haemagglutinin which show multiple basic amino acids (arginine and lysine). Moreover some strains gave rise to a 75% mortality rate in embryonated eggs by the third day p.i. (365/A97 and 373/A97), while the strains 326/A97 and 330/A97 gave rise to a 100% mortality rate in inoculated embryonated eggs after 48 h and 24 h respectively.

Taking into consideration all the data in our possession up to now, it can be concluded that the isolated strains of avian influenza virus are extremely pathogenic and are closely related if not, indeed, identical. These viruses are spreading slowly through the rural farms in the Veneto region probably aided by the retail trade of live birds by breeders owning infected animals. Nor can the fact that rurally bred birds in the absence of biosecurity conditions may have had numerous occasions for contact with waterfowl be ignored. However, although the Veneto region is one of the highest density poultry-producing regions, the virus has not spread to the commercial farms and so it can be deduced that the safety measures and checks carried so far have reduced the spread of this dangerous influenza virus.

The situation is being carefully monitored by the Istituto Zooprofilattico delle Venezie, the Italian Reference Centre for Avian Influenza Viruses of University of Naples Federico II, The Istituto Superiore di Sanità - Veterinary Laboratories, the Italian Reference Centre for Human Influenza Viruses, and by the Ministry of Health. Finally, it should be stated that ring vaccination has not been used.

Table 1. Epidemiological situation of Avian Influenza in Italy (Veneto region) during 1997

No	Outbreak suspected	Virus isolation	Depopulation	Town	chickens	turkeys	guinea fowl	duck	quail	pigeon	pheasant	geese
1	07/10/97	27/10/97	27/10/97	Dueville (VI)	9/14*	0/2		0/16				0/2
2	15/09/97	30/10/97	31/10/97	Vittorio Veneto (TV)	160/1480		50/600	0/1100		0/25	0/1	0/2
3	06/10/97	11/11/97	02/11/97	Talmasson (UD)	32/40							
4	05/11/97	18/11/97	23/11/97	Crepaldo Eraclea (VE)	23/50	0/5		0/16		0/12		
5	10/11/97	18/11/97	20/11/97	Giacciano con B (RO)	42/84		0/4	0/52	0/98	0/8		
6	20/11/97	24/11/97	26/11/97	Pozzoleone (VI)		423/1500	0/100	0/600	0/150		0/30	
7	12/11/97	25/11/97	26/11/97	Boion di Campolongo Magg. (VE)	30/30		70/70	0/60				10

*Number dead of total number of species

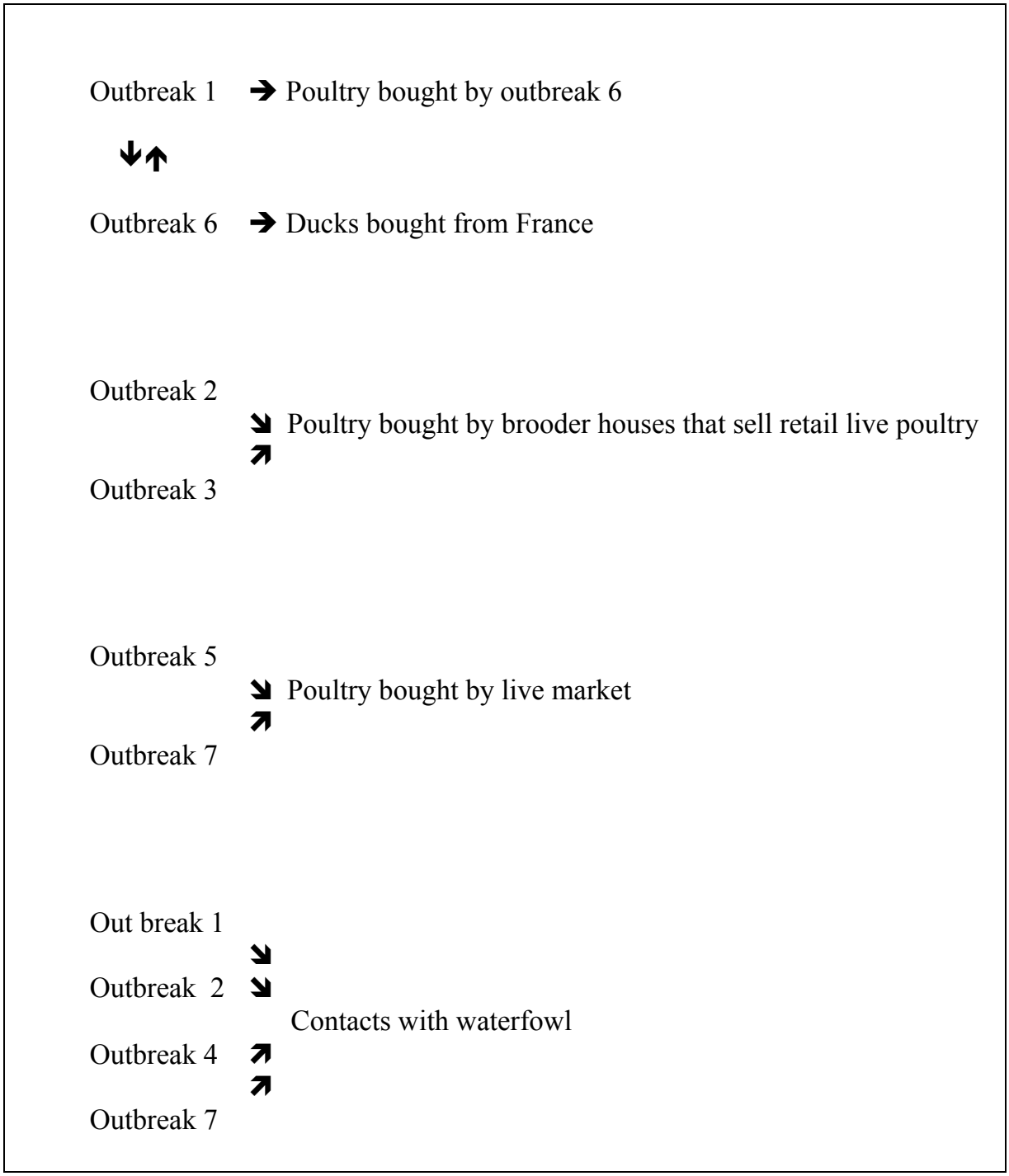
Note: The numbers are a estimations of the Veterinary Regional Service.

Table 2. Virological data of avian influenza viruses isolated in Italy during 1997

No	Strain	Isolation source	HI titre	Subtype	IVPI	H cleavage site
1	312/A97	pool of chicken organs	1:1024	H5N2	3	PQRRRKKR⇩GLF
2	326/A97	pool of chicken organs	1:680	H5N2	3	PQRRRKKR⇩GLF
2	330/A97	pool of guinea-fowl organs	1:1128	H5N2	3	PQRRRKKR⇩GLF
3	367/A97	pool of chicken cloacal and tracheal swabs	1:1280	H5N2	2,98	PQRRRKKR⇩GLF
4	365/A97	pool of hen organs	1:1280	H5N2	2,98	PQRRRKKR⇩GLF
5	373/A97	pool of chicken organs	1:1280	H5N2	3	PQRRRKKR⇩GLF
6	382/A97	pool of guinea-fowl organs	1:640	H5N2	3	PQRRRKKR⇩GLF
7	392/A97	turkeys cloacal swabs	1:640	H5N2	2,94	PQRRRKKR⇩GLF

Figure 1 Geographical distribution of influenza outbreaks in Northern Italy during October - November 1997

Figure 2. Epidemiological interactions among AI outbreaks in Veneto, Italy 1997



COUNTRY REPORTS FOR 1996-1997

NEWCASTLE DISEASE

The following countries made simple statements of no Newcastle disease outbreaks during 1996-1997:

Greece
Spain

A presentation was made for Portugal but no manuscript was received.

NEWCASTLE DISEASE IN ITALY : 1996 - 1997 SITUATION

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The 92/66 EEC directive was acknowledged on 15 November 1996 and the Avian Virology Laboratory of Istituto Zooprofilattico of Padua has been officially recognised as the National Reference Laboratory of the Newcastle Disease. Several PMV-1 strains from different Italian regions were submitted for diagnosis or confirmation.

Assessment of the virulence of NDV isolates for chickens is made by ICPI test ; for biological and antigenic characterisation plaque formation, MDT/MLD calculation and HI tests with three monoclonal antibodies, kindly supplied by the EU Reference Laboratory, Weybridge, were used. Further typing and confirmation of pathogenicity indices were determined by the EU Reference Laboratory.

During the last two years (October 1995 - November 1997) all the NDV outbreaks officially recognised in Italy have been caused by pigeon PMV-1 strains. In Table 1, data concerning these outbreaks are summarised.

In most cases, nervous signs and high mortality were recorded. The origin of the outbreaks was not determined, their spread was caused by movement of animals.

The ostrich PMV - 1 strain should be noted: the virus had been isolated from brain and intestine of a four-month-old ostrich which died suddenly. The animal lived with two other subjects which did not show any signs or seroconversion. Also in this case the origin of the PMV-1 infection was not determined, as the virological and serological investigations of the flock of origin had given negative results; therefore direct or indirect contact with infected pigeons is assumed.

Similarly in the case of the sparrow-hawk isolate, the origin has to be considered to be infected pigeons, as the subject, the only one sick in the group of 10 animals, had eaten pigeon meat.

There were no confirmed cases of disease in commercial chicken flocks, perhaps because of vaccination plans implemented on a voluntary basis.

The Veterinary Service of the Ministry of Health adopted all the measures provided for by the Veterinary Police Regulation and by 92/66/EEC, the Newcastle disease

control directive: slaughter of all the animals in infected holdings and disposal off the carcasses and litters, disinfection, establishment of protection zones and surveillance zones, prohibition of fairs, reinforced vaccinations etc.

Table 1. Newcastle disease viruses isolated in Italy October 1995 to November 1997.

Case N°	Date	Region, Province	Type of flock	Virus Isolated	Species	Pathogenicity indices	mAb Group
1	13/11/95	Veneto, Verona (NE)	backyard flock	P-PMV1	pheasants	ICPI=1,51 IVPI=0,92	P
2	6/2/96	Veneto Vicenza (NE)	hobby flock	P-PMV1	turtle doves	ICPI=1,32 IVPI=0,45	P
3	1/4/96	Lombardia Piacenza (N)	hobby flock	P-PMV1	pigeons	ICPI=1,20 IVPI=0,34	P
4	18/4/96	Veneto Venezia (NE)	hobby flock	P-PMV1	pigeons	ICPI=1,025 IVPI=0,00	P
5	7/8/96	Veneto Verona (NE)	hobby flock	P-PMV1	pigeons	ICPI=1,175 IVPI=N.D.	P
6	9/1/97	Lazio Roma (C)	backyard flock	P-PMV1	ostrich	ICPI=1,12 IVPI=N.D.	P (+ve mAb U32)
7	25/2/97	Veneto Verona (NE)	backyard flock	P-PMV1	pigeons	ICPI=1,44 IVPI=N.D.	P
8	15/5/97	Lazio Viterbo (C)	faunistic firm	P-PMV1	sparrow hawk	ICPI=1,14 IVPI=N.D.	ND(+ve. mAb 617/161)
9	29/10/97	Piemonte Cuneo (NW)	hobby flock	P-PMV1	pigeons	ICPI=1,32 IVPI=N.D.	ND (+ve mAb 617/161)
10	1/7/97	Lazio Roma (C)	backyard flock	P-PMV1	pigeons	ICPI=1,11 IVPI=N.D.	ND (+ve. mAb 617/161)
11	8/10/97	Veneto Verona (NE)	pigeon	P-PMV1	pigeons	ICPI=1,06 IVPI=N.D.	ND(+ve. mAb 617/161)

ICPI - intracerebral pathogenicity index; IVPI - intravenous pathogenicity index, ND - not done

N - North, NW - Northwest, NE - Northeast, C - Central, S - South.

NEWCASTLE DISEASE IN AUSTRIA 1996-1997

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Introduction

ND is seen only occasionally in Austria, and the two outbreaks which occurred in 1993, one in May, in turkeys and the other, in June, in broiler chickens which had not been vaccinated, were the first for some five years (Kissling, 1994). No outbreaks occurred during 1994-1995.

Outbreaks 1996 - 1997

Four outbreaks were confirmed in 1996 (Table 1) and two in 1997 (Table 2).

Table 1 Confirmed outbreaks in Austria in 1996

Date	Province	District	Number and type of birds	ICPI
18.01.96	Lower Austria	Wien-Umgebung	101 racing pigeons	1.26
08.02.96	Styria	Voitsberg	30 backyard chickens	1.88
15.04.96	Lower Austria	Raden	40 pigeons, 3 pheasants, 3 quail, 7 chickens	1.55
07.10.96	Lower Austria	Wr Neustadt Land	15 pigeons 8 pheasants	1.26

Table 2 confirmed outbreaks in Austria in 1997

Date	Province	District	Number and type of birds	ICPI
05.11.97	Vienna	10	23 ornamental pigeons	1.39
28.12.97	Upper Austria	Freistadt	220 pigeons, 7 quail, 5 geese, 1 duck, 10 turkeys, 20 bantams	1.28

The Community Reference Laboratory confirmed viruses isolated from the three outbreaks in Lower Austria in 1996 and the outbreak in Vienna in 1997 to be the pigeon panzootic variant (PPMV-1) showing monoclonal antibody [mAb] binding pattern P, although the virus from the first 1996 outbreak failed to cause binding of one of the mAbs which normally binds to PPMV-1 isolates. Virus from the backyard chickens in Styria was more classical velogenic ND virus causing binding pattern B with the mAb panel. The Upper Austria virus has not yet been tested with the full panel but preliminary tests indicate it too is a PPMV-1 virus.

Kissling, R. (1994) Current Newcastle disease situation in Austria. *Proceedings of the Joint First Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Communities* pp 44-45.

NEWCASTLE DISEASE : CURRENT SITUATION IN FRANCE FROM OCTOBER 1995 TO OCTOBER 1997

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From October 1995 to October 1997, a total of 74 suspected cases was investigated from poultry, pigeons, caged birds and wild birds. Sixty nine cases were negative (Tables 1 and 2). Only five cases were positive : one was a PMV-2 virus and the others PMV-1 (Table 3). Of the PMV-1 isolates, two were pathogenic with an ICPI >0,7 : the first was in the Ile de la Réunion which was submitted to the European Union Reference Laboratory for characterisation (and showed group B mAb binding), the other was a variant PPMV1 virus isolated from a pigeon reared in a back yard.

Table 1 : Suspected cases investigated with negative results from October 1995 to December 1996

Month Year	Species	N° of Suspect cases
Oct. 95	Fowl	3
	Budgerigar	1
Nov. 95	Pigeon	1
Dec. 95	Turkey	2
Jan. 96	Pigeon	2
	Fowl	3
	Cage bird	1
Feb. 96	Turkey	2
	Fowl	3
	Pigeon	2
	Seagull	1
March 96	Pigeon	3
	Fowl	5
May 96	Pigeon	1
	Guinea fowl	1
June 96	Fowl	2
July 96	Fowl	3
	Pigeon	2
	Canary	1
	Pheasant	1
Aug. 96	Fowl	1
Sept. 96	Fowl	2
	Turkey	1
Oct. 96	Fowl	1
Nov. 96	Pigeon	1
Dec. 96	Turkey	1

Data from 3 diagnostic laboratories

Table 2 : Suspected cases investigated with negative results from January 1997 to October 1997

Month Year	Species	N° of Suspect cases
Jan. 97	Canary	1
March 97	Turkey	1
April 97	Pigeon	2
	Turkey	2
	Fowl	3
May 97	Fowl	1
	Guinea fowl	1
	Pigeon	1
June 97	Fowl	1
July 97	Fowl	2
	Parrot	1
Sept. 97	Fowl	1
	Pigeon	1
Oct. 97	Fowl	1
	Turkey	1
	Pigeon	1
	Backyard	1

Data from 3 diagnostic laboratories

Table 3 : Avian PMV viruses isolated in October 1995 to October 1997

Country (Department)	Month Year	Species	Type of virus	ICPI	Case history
32	Dec. 95	Turkey	PMV-1	0,34	/
Reunion	May 96	Chicken	PMV-1	1,87	Mortality
45	Aug. 96	Pheasant	PMV-1	0	Mortality 25%
Nouvelle Caledonie	Sep. 96	Cage bird	PMV-2	/	Mortality 35 %
28	Sep. 97	Pigeon	PMV-1	1,28	Mortality

NEWCASTLE DISEASE SITUATION IN BELGIUM

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Velogenic Newcastle disease was diagnosed twice in 1997. The first case was observed in June and the second in October.

Case Number 1:

A velogenic PMV1 (PMV1-97/115VB) virus was isolated from backyard chickens. The ICPI of the strain was: 1.99.

Serological typing using monoclonal antibodies was performed in Weybridge laboratory by Ruth Manvell and Dennis Alexander and showed that the virus belongs to the B group of velogenic viruses.

The infected flock was located in Neufchâteau, province of Luxemburg.
The total number of animals in the outbreak was 263 birds: 200 quails, 51 chickens, 4 guinea fowl, 4 peacocks, 2 turkeys and 2 geese.
A stamping-out policy was implemented.
The source of the infection is unknown.

Case Number 2:

A velogenic virus was isolated from the cerebrum of a dead pigeon and characterised as belonging to the pigeon group of velogenic Newcastle disease viruses using monoclonal antibodies. The ICPI of the strain was 1.40.

The hobby flock of homing pigeons was located in Jambes, province of Namur. The total number of pigeons in the outbreak was 83 of which 43 were clinically affected and 20 died.

No other cases were diagnosed. This favourable epizootiological situation could be a result of the compulsory vaccination policy. However, we need to be careful in our evaluation of the real situation as we can only isolate viruses from samples that are sent to our laboratory.

NEWCASTLE DISEASE: SITUATION IN THE NETHERLANDS 1996-1997

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Outbreaks in 1996

On June 12 1996, NDV was isolated from an ornamental pigeon purchased from a local market. The isolate has an ICPI of 1.0. The sequence at the fusion protein cleavage site was determined as RRQKR-F. Results define the isolate as a mesogenic PMV-1. As a consequence the outbreak was reported to the OIE on June 28 1996. The outbreak occurred in Landgraaf, a small city in the Southern part of the Dutch province Limburg, near to the German border. All birds at the premises were killed.

On July 18 1996, a lapwing was send to our laboratory. The lapwing came from a revalidation centre for wild birds in Anjum located near the coast in the province Friesland. The centre kept 286 birds of different species including lapwings (*Vanellus vanellus*), ducks, gees, ornamental chickens, pigeons and birds of prey. The lapwings in the centre with an age of 4-6 weeks showed respiratory and nervous signs. At autopsy no abnormalities were observed. Eggs inoculated with allantoic fluid of the first passage died within 24 hours, probably because of bacterial contamination. Filtered allantoic fluid however contained a haemagglutinating virus. Haemagglutination was inhibited with mAbs 7B7, 8C11 and 4D6 but not with mAb 7D4 that is specific for the La Sota strain (mAbs were provided by Dr. Meulemans). The isolate had an ICPI value of 1.4 and the sequence at the fusion protein cleavage site was determined as KRQKR-F. The outbreak was reported to the OIE on July 31. All birds at the premises were killed and the site cleaned and disinfected. No commercial poultry were kept in vicinity of the revalidation centre. The centre had no quarantine facility and wild birds probably introduced the disease.

On August 8 1996 dead wild ducks were sent to the institute for diagnosis of botulism. Dead birds were first found Lemsterland in the province of Friesland in week 31 and another 10 birds the next week. However, no bacteria were isolated from dead birds and therefore organ suspension were send by the Department of Bacteriology for virus isolation. A haemagglutinating virus was isolated. Haemagglutination was inhibited with mAbs 7B7, 8C11 and 4D6, but not with mAb 7D4. Using primers specific for NDV a 364-kb fragment of the genome was amplified. However, no amplification was obtained using primers specific for

virulent or lentogenic NDV viruses. The sequence at the cleavage site was determined as KRQKR-F. The sequence around the cleavage site was identical to the virus that was isolated from lapwings and therefore we tentatively conclude that both viruses are identical. The ICPI of the isolate was 1.4.

Twelve six-week-old Pekin ducks that were inoculated intravenously with the isolate did not show any clinical signs. Nevertheless although all ducks scored positive in the NDV-ELISA 28 days after infection. Two of 9 three-week-old sentinel chickens showed clinical signs and died between 11 and 14 days after the start of the contact exposure. Three of the remaining 7 chickens had an antibody titre in HAI tests at the end of experiment. Therefore, the Pekin ducks had shed virus into the environment. All 12 six-week-old chickens that were intravenously infected became ill at 4 days after infection. Chickens showed depression and nervous signs. Eight of 12 chickens died between day 12 and 25. The results indicate that infected ducks can spread NDV to other birds but do not allow the conclusion that the wild ducks died because of NDV infection.

Outbreaks in 1997

On August 12 1997, allantoic fluid was sent to our laboratory for NDV differentiation. Virus was isolated during the second passage from organ suspensions of a pigeon. At this passage level, all embryos died within 3 days. The virus was characterised as NDV using the agar gel precipitation test. The ornamental pigeon that was housed in village Wamelen in the province Gelderland was recently imported form Indonesia. A 364-kb fragment was amplified from RNA isolated from the allantoic fluid and primers specific for NDV. No amplification was obtained when primers specific for virulent or lentogenic NDV were used. The sequenced at the cleavage site was determined as RRQKR-F. As a consequence all birds at the premises were killed and the site disinfected. The haemagglutinating activity of the isolate was not inhibited with mAb 617/161 (courtesy D. Alexander) specific for pigeon viruses.

NEWCASTLE DISEASE - SITUATION IN GERMANY 1995 - 1997

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There have been many outbreaks of ND in Germany in recent years (Table 1).

Table 1. Outbreaks of ND in Germany 1992-1997

Year	Number of outbreaks
1992	2
1993	181
1994	179
1995	28
1996	2
1997	0

However, the situation has changed completely since 1995 and no case was detected in 1997. The outbreaks in former years occurred primarily in small and hobby flocks and the most common means of transmission was the purchase of birds.

Only 1 of the 28 outbreaks in 1995 occurred in commercial birds, on a farm with 38000 broilers. The other outbreaks affected small rural and hobby flocks with ornamental birds or backyard poultry. One outbreak occurred in a pigeon house.

In the past vaccination against ND was compulsory only for flocks with more than 200 animals. In the new version of the "Legal Decree for Protection against Fowl Plague and Newcastle Disease" of the 21st December 1994 vaccination against ND was made compulsory for all chickens and turkeys, regardless of the size of the flock. This policy has improved the situation and we have observed a noticeable reduction of ND.

In 1996 we had only two confirmed cases, one in a small pheasant farm and one in backyard poultry.

In 1997 no outbreaks of ND were reported.

Most virus isolates from the confirmed outbreaks in 1995-1996 were of high virulence with ICPI values of about 1.8 or more (Table 2). Further characterisation at the EU Reference Laboratory in Weybridge by monoclonal antibodies showed that they had a binding pattern similar to those seen recently in Europe but distinct from the group known as NE virus. These results confirmed our suspicion that the virus of the epizootic in Germany in 1993 until 1996 has undergone a certain alteration which we detected had with our own monoclonal antibodies.

Table 2. Biological characteristics of PMV-1 isolates

Virus	1995	1996	1997
velogenic	26	2	-
pigeon PMV-1	2 + 22	23	20
lentogenic	68	5	8

Two of the confirmed ND cases in 1995 were caused by pigeon type PMV-1, one in a pigeon house and one in backyard poultry. Other pigeon type PMV-1 isolates submitted to the National Reference Laboratory came from domestic or wild pigeons. They were identified by HI test with monoclonal antibodies from our own laboratory and with 617/161. The latter one was kindly provided by Dr. Alexander.

Some viruses which we received from regional diagnostic laboratories proved to be lentogenic and the suspicion of ND could not be confirmed. Lentogenic viruses were obtained from different species (Table 3).

Table 3. PMV-1 isolates with ICPI below 0.7

isolated from:	1995	1996	1997
chicken	23	4	5
turkey	5	-	1
duck	3	-	-
pigeon	13	1	1
fancy birds	24	-	1
Totals	**68**	**5**	**8**

All lentogenic isolates had very thermolabile haemagglutinins. Most isolates from chickens, turkeys and pigeons were inhibited by the monoclonal antibody 7D4. This leads to the conclusion that most of these lentogenic isolates are vaccine virus of La Sota type. The use of live vaccines with La Sota virus is very common in Germany and one such vaccine was allowed to be used for pigeons until 1996. The high number of isolations of lentogenic viruses in 1996 may be caused by the increased activity of the regional laboratories as a result of the ND epizootic in years before.

NEWCASTLE DISEASE - COUNTRY REPORT FOR NORWAY 1996-1997

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As in previous years flocks on all parent poultry farms have been tested for PMV-1 antibodies as a part of a testing scheme of the breeding stock in connection with a health certification and documentation programme. All flocks in import quarantine have also been tested. In 1996 a total of 156 flocks and comprising approximately 9,000 samples were tested (60 samples per flock), and six flocks representing one farm were found positive. Seroreactors have been detected on this farm for several years, and in 1994 a strain of lentogenic PMV-1, Weybridge monoclonal group C2, was isolated.

At the end of December 1996 a disease outbreak occurred in a layer flock with 45 weeks old birds on an island on the south western coast of the country. The main symptoms were respiratory distress, anorexia and sharply declining egg production. Within five days after the first observation of disease approximately 50% of the 1,800 hens in the flock had died. Most of the remaining animals were also sick, and they were killed and buried. PMV-1 was isolated and the isolate was characterised in Weybridge. An ICPI of 1,87 was obtained and the virus was placed in mAb group C1.

On testing of all the other flocks on the island, antibody reactors were found in 19 out of 24 farms, in most cases with a prevalence close to 100%. No disease or drop in egg production had been observed in these flocks. It turned out that all the antibody positive flocks originated from the particular farm infected with lentogenic virus recorded in 1996 and earlier. The owners of the five seronegative flocks on the island had got their birds from other chick suppliers. Tracer chicks were placed in seropositive flocks on two farms located in the close neighbourhood to the outbreak farm. However, attempts to isolate virus from these chicks gave negative results.

Also the chick-producing plant which had delivered the outbreak flock turned out to have a high prevalence of seropositive birds. No disease or drop in egg production with possible relevance to PMV-1 infection had been recorded on this farm. Virus isolation attempts from test chicks brought into the flocks on this farm were negative.

Ten waterfowl and seabirds shot near the outbreak farm were virus and antibody negative. A goose flock which was kept outdoors all the year had one seroreactor (HI-titre 1:16) among 40 birds.

The farm where the ND outbreak occurred is located close to a bay with a rich fauna of waterfowls and seabirds. Gulls, crows and small birds were frequently seen in an open outer room of the animal building, feeding on wasted fodder. The birds had no access to the food store. Dead birds were occasionally found in this room. Through person traffic there was a close contact between this contaminated area and the hens. Thus, when also taking the mAb type of the virus into consideration, it seems reasonable to believe that the outbreak was of wild bird origin.

During 1997 168 parent flocks and approximately 10,000 blood samples were tested as a part the health certification programme and in connection with import quarantine. Apart from the seropositive flocks on the two abovementioned farms one additional positive flock was found. This was a broiler parent flock located in the inland of the south eastern part of the country. Virus isolation trials with tracer chicks brought into the farm were negative, and no PMV-1 antibodies were detected in broiler flocks supplied by this particular farm. The origin of the infection remains unknown. Contaminated vaccines as a possible source have been discussed.

The persistence of the apathogenic or lentogenic PMV-1 in some parent farms certainly interferes with the surveillance, control and diagnosis of ND. So far this has been a problem limited to one single county and especially concerning one particular large plant.

Taking economic consequences for this farm into consideration and in order not to impair the industry in the most important region for egg production in the country too much, the veterinary authorities have allowed sale of hatching eggs and chicks from the plant, but only within the county. In attempts to eliminate the virus without stopping production, isolation and hygienic measures have been imposed. However, the farm comprises many houses located close together and has a continual production with flocks at different ages, and so far the efforts have not been successful.

NEWCASTLE DISEASE: SITUATION IN SWEDEN OCTOBER 1995 - 1997

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No clinical outbreak of Newcastle disease (ND) occurred during 1996 in Sweden. At the end of 1995 during the same week as we held our last meeting in Brussels and in October 1997 we had outbreaks of ND for the first times since 1956.

Outbreak 1995

The ND outbreak in October-November 1995 occurred in the largest broiler breeding company in Sweden supplying day-old broiler chickens to about 45% of the market for poultry meat production. The outbreak was limited to the central unit of the company with the hatchery and parent flocks in 12 houses producing hatching eggs. Notably, there are only 50-200 metres between the different houses in the central unit and 200 metres between the hatchery and the closest poultry house.

Clinical signs of disease with a dramatic loss of egg production were seen at the end of October in one flock and a serological test revealed high titres of antibodies against PMV-1. The same day sera from flocks in the adjacent houses tested in the mandatory routine surveillance programme were found to have antibodies against PMV-1 as well. These flocks had not started egg production and had not shown any sign of disease.

Newcastle disease virus was isolated. The ICPI was 1.78, high compared to the relative mild symptoms. The isolate was closely related to the Danish isolate from back yard flocks (BYFs) in September.

All flocks in the central unit of the company were killed and destroyed as several showed serological evidence or signs of disease. The hatchery was closed and 1.7 million eggs and chickens were destroyed. The broiler flocks (26) with birds that had been delivered from the hatchery one week before the hatchery was put under restrictions were kept under close observation and tested serologically before slaughter. No clinical signs were registered in any of these flocks and all samples were seronegative for PMV-1.

A large clinical and serological investigation was carried out in both the protection and surveillance zones to find a source of infection or spread of the disease. All commercial flocks, contact flocks and almost all BYFs were tested. No commercial flocks outside the central unit were infected, but 21 BYFs had some birds with

specific antibodies against PMV-1. Six of them had serological evidence of active infection, but virus could not be isolated. None of the flocks had a history of disease. The reason for testing so many BYF (>500) was the early unexpected finding of seropositive birds in this population. The results indicate that several BYFs in southern Sweden have been in contact with PMV-1 of unknown origin.

No source of infection could be found. All possible contacts were investigated and found free from infection. As the isolate of NDV was closely related to Danish isolates we suspect a common source possibly wild migrating birds. The surroundings of the establishment are popular resting places for migrating birds. Seventy-six wild birds of different species were shot and samples for virus isolation were taken but no virus could be found.

Outbreak October 1997

Suspicion of disease was reported from a farm with both broilers (3,000) and layers (850). The layers had totally stopped laying eggs and 1,000 of the broilers laid down on the floor with head tremors. Blood samples showed high titres of antibodies against PMV-1 and one week later the ICPI of the isolated virus was found to be 1.86. The isolate was closely related to the other Nordic isolate in 1996 and 1997, first with monoclonal binding pattern technique and later with gene sequencing.

All birds were killed and buried and searching for contacts started immediately. All contact flocks were investigated both clinically and serologically and all were found free of ND. All other flocks in the protection and surveillance zones were visited several times during the restriction period which ended on the 30th November. Several layer flocks showed decrease in egg production but were serologically free from antibodies against PMV-1. No other sign of disease were seen and the ND outbreak had not spread from the index farm.

Thirteen broiler flocks were tested according to directive 91/494 EEC five days before transported to slaughter with 30 cloacal and 30 tracheal swabs. No HA-agent were detected from these flocks.

COUNTRY REPORT - NEWCASTLE DISEASE IN FINLAND

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PMV-1 was isolated on four different occasions in 1996. According to council directive 92/66/EEC article 1 the definition for ND applied in two of the cases when the virus was detected in captive birds. The virus isolations and characterisation as PMV-1 were done in the National Laboratory. The typing of the isolates with monoclonal antibodies and the intracerebral pathogenicity index test were done at the EU Reference Laboratory in the UK.

The first PMV-1 isolate in 1996 was from a pigeon in an aviary at the University of Oulu in Northern Finland. The aviary had received pigeons captured in Helsinki at the end of March. Three days after arrival one pigeon was found dead. Within three weeks 23 pigeons out of 95 died. Gastrointestinal and nervous signs were observed.

PMV-1 pigeon type was isolated. The ICPI was 1.32. When the diagnosis was confirmed all birds were killed on the spot and destroyed together with the eggs. Disinfection of the premises was carried out according to Council Directive 92/66/EEC.

The second PMV-1 isolate that was classified as ND was from a goosander at the Helsinki Zoo. The young bird was kept in a separate building for rescued nestlings. Before death the bird had shown nervous signs. This time the PMV-1 was of ”classical” type, mAb group C1, and had an ICPI of 1.37. This same strain has been isolated from pheasants in Denmark at about the same time.

Later in 1996 PMV-1 was isolated twice from feral pigeons in Helsinki. The ICPI of these pigeon type isolates was around 1.4.

In 1997 PMV-1 was isolated once, from a mallard duck. This isolate was of low virulence and placed in the H mAb group.

Annual screening for ND in breeding flocks (5457 samples in 1996) has been done. No positive samples were detected

NEWCASTLE DISEASE - CURRENT SITUATION IN DENMARK OCTOBER 1995 - OCTOBER 1997.

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Definition.

The definition of Newcastle Disease (ND) in Denmark conforms to the definition presented in the EU Directive 92/66/EEC introducing Community measures for the control of Newcastle Disease.

Diagnostic methods.

The diagnostic methods follow the recommendations in the above mentioned EU Directive. No alterations of the diagnostic procedures have been made since the last meeting but methods for RT-PCR amplification and nucleotide sequencing of the PCR products have been established. Until now these methods have been applied to virus isolates harvested from inoculated embryonated eggs.

Epidemiological situation.

In the period from 1st October 1995 to 31st December 1995 the laboratory received a total of 163 submissions of poultry for virological examination. Newcastle disease virus was isolated from 14 flocks (Table 1). The ICPI of these APMV-1 isolates varied between 1.26 and 1.81 (mean = 1.65). The size of the flocks from which NDV was isolated was estimated as less than 100 birds in all cases. With the exception of two flocks of imported ostriches and emus in quarantine, the remaining flocks were back yard poultry. Fowls, ducks, geese, turkeys, pigeons, ostriches and game birds were represented in the flocks. All APMV-1 isolated from these outbreaks were characterised with a panel of monoclonal antibodies by the EU Reference Laboratory (Table 2). All but one isolate appeared to have a unique binding pattern with significant differences to APMV-1 strains formerly isolated in the EU.

In fowl the infection was associated with increased mortality, reduced egg production and respiratory illness while clinical signs were not significant in infected ostriches and web-footed birds. The binding pattern of the remaining isolate suggested it being a mixture containing pigeon APMV-1. Control measures were raised in accordance with EU Directive 92/66 and ratites in quarantine were rejected.

In 1996 the laboratory received 384 submissions for virology and NDV was

isolated from five flocks of poultry (Table 1). One outbreak in a large and one in a small table egg producing flock together with one outbreak in caged partridges were handled according to the directive. The two remaining ND outbreaks in 1996 took place in free-range pheasants to which the directive did not apply. The ICPI of the 1996 APMV-1 isolates ranged between 1,64 to 1.88 (mean = 1.73). Characterisation by monoclonal antibodies at the EU reference laboratory revealed that they belonged to the C1 antigenic group (Table 2).

Neither in 1995 nor in 1996 could the source of infection be identified but transmission by feral birds is thought to be likely.

In 1997 there were 302 submissions for virology (up to December). So far no outbreaks of ND have been ascertained in Denmark in 1997.

By means of sequence analysis of a RT-PCR amplified segment of the F0 viral protein it was found, that APMV-1 strains representative for the 1995 and 1996 Danish ND outbreaks had a deduced amino acid sequence at the cleavage site which corresponds with sequences of virulent APMV-1 strains.

In order to demonstrate absence of antibodies against APMV-1 serum samples from commercial poultry parent stock are tested routinely. In 1997 positive titres against APMV-1 were detected in six flocks of broiler parent stock and one flock of broilers with no clinical signs of disease. Furthermore contamination with low virulent live APMV-1 vaccine strains (La Sota-like and B1-like) has been demonstrated in a number of live avian vaccines used in Denmark in 1997. Three of the flocks with APMV-1 positive titres had been vaccinated with these contaminated batches of vaccine. A low virulent APMV-1 strain (La Sota/Clone 30-like) was isolated from one parent flock (ICPI = 0.0), no virus was isolated from five flocks, while a APMV-1 isolate from the remaining flock is still under investigation. Therefore there is a theoretical possibility of APMV-1 from contaminated vaccines circulating in the Danish commercial poultry population.

References

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Table 1. NDV isolates in Denmark Oct. 1995 - Oct. 1997

1995					
Date	***Identification***	***Host***	***Flock size***	***ICPI***	***Species in the flock***
3 Oct.	DK/76922/95	Fowl	< 50	1.68	Fowls
16 Oct.	DK/77280/95	Duck	< 50	1.64	Fowls & ducks
16 Oct.	DK/77281/95	Goose	50-100	1.65	Fowls, geese & turkeys
19 Oct.	DK/77346/95	Pigeon	50-100	1.63	Fowls, ducks, pigeons & turkeys
24 Oct.	DK/77428/95 [1)]	Ostrich		1.65	
10 Nov.	DK/77961/95 [1)]	Ostrich	50-100	1.69	Ostriches & emus
14 Nov.	DK/78051/95 [1)]	Ostrich		1.63	
26 Oct.	DK/77540/95	Fowl	< 50	1.65	Fowls
2 Nov.	DK/77721/95 [2)]	Fowl	< 50	1.26	Not known
13 Nov.	DK/77976/95	Fowl	< 50	1.70	Fowls
14 Nov.	DK/78046/95	Fowl	< 50	1.68	Not known
14 Nov.	DK/78052/95	Fowl	50-100	1.68	Fowls
15 Nov.	DK/78096-7/95	Fowl & Duck	< 50	1.64	Fowls & ducks
15 Nov.	DK/78101/95	Ostrich	Not known	1.81	Not known
20 Nov.	DK/78171/95	Partridge	< 50	1.70	Game birds, pigeons, turkeys & fowls
20 Nov.	DK/78174/95	Fowl	50-100	1.65	Fowls & ducks
1996					
9 Aug.	DK/75317/96	Fowl	< 50	1.75	Fowls
13 Aug.	DK/75374/96	Fowl	9,000	1.76	Fowls
26 Aug.	DK/75661/96	Pheasant	10,000	1.88	Pheasants (free-range)
3 Sept.	DK/75916/96	Pheasant	8,000	1.63	Pheasants (free-range)
4 Sept.	DK/75965/96	Partridge	Not known	1.64	Pheasants & partridges (caged)

[1)]DK/77428/95, DK/77961/95 and DK/78051/95 were isolated from the same flock of ostriches. [2)]Untyped isolate

Table 2. Characterisation by monoclonal antibodies of NDV isolates from Denmark Oct. 1995 - Oct. 1997 in comparison with isolates from other European countries 1991-95.

Country	*Year*	*No of isolates*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	*v*	*w*	*x*	*y*	*z*	*£*	*$*
Denmark	1995	15 [1)]	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	+
Sweden	1995	1	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	+
Switzerland	1995	1	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	NT	+	NT	+	-	+	-	+	-	+
Denmark	1996	5	+	NT	NT	NT	+	-	+	+	-	-	+	-	+/-	NT	+	+	+	+	+	+	+	+	-	-	NT	+	-	+
Germany [2)]	1993-95	?	-	-	+	-	?	-	+	-	-	-	+	-	+	+	+	+	-	-	+	+	+	-	-	-	-	+	-	+
EU [3)]	1991-95	?	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	-	-	+	-	-	-	+	-	+
Portugal	1991-94	?	+	-	+	+	+	-	+	+	-	-	+	-	+	+	+	+	-	-	+	+	+	-	-	-	-	+	-	+

[1)] One untyped APMV-1 isolate not included.
[2)] Pigeon variant of APMV-1 isolates not included.
[3)] Continental member states of the EU including Germany, Netherlands, Belgium, Luxembourg, France and Spain.
The mAbs used were: - a-i: 14, 32, 38, 86, 424, 445, 479, 481 and 688 prepared against NDV Ulster 2C (Russell and Alexander, 1983); j-v: U11, U23, U32, U45, U49, U55, U57, U67, U68, U69, U70, U79 and U85 prepared against Ulster 2C (Collins and Alexander , unpublished); w-z, £ and $: 3/617, 38/617, 43/617, 54/617, 161/617 and 165/617 prepared against isolate PMV 1/pigeon/England/617/83 (Collins et al. 1989). The binding patterns were obtained by assessing the ability of mAbs to bind (+) to Vero cels infected with the isolate using an indirect immunoperoxidase monolayer assay.

REPORT OF THE IRISH NATIONAL REFERENCE LABORATORY FOR NEWCASTLE DISEASE FOR 1996 AND 1997

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1996

Ireland maintained a non-vaccination policy for Newcastle Disease in poultry. Vaccination of racing pigeons for PMV-1 was compulsory.

During the year there were 7 isolates of the pigeon variant strain PMV-1 from pigeons.

Three isolations of an apathogenic group L, 'Loon-like strain' of PMV-1 (ICPI 0.00) were made following seropositive serological reactions in 2 commercial turkey flocks and one breeding turkey flock, identified during surveillance testing.

1997

There was one outbreak of Newcastle disease in Co. Cavan in March, in a unit of 20,000 9-day-old imported broilers. High mortality had commenced at 5 days of age and reached 4,000 daily by 11 days. The history indicated that a group of 500 of the birds had been vaccinated with Hitchener B1 as day-olds and these were inadvertently mixed with 19,500 non-vaccinated birds prior to transportation to the site.

Clinically the flock presented with high mortality, the birds collapsing and dying with no prior clinical signs. There was no evidence of respiratory signs, diarrhoea or nervous signs in the rest of the flock. Carcasses were congested, with pale livers and haemorrhages in the proventriculus. Histopathology demonstrated focal necrosis of the liver and proventriculus, acute renal tubular necrosis and encephalitis.

PMV-1 virus was isolated, and confirmed as the C1 strain of PMV-1 similar to that found in Northern Ireland and Great Britain. The ICPI was 1.8.

The flock was restricted and the birds slaughtered. Investigations in the 3k protection and 10k surveillance zone revealed no further evidence of disease.

This flock had been imported as day-olds from another member state with current outbreaks of Newcastle disease.

A voluntary vaccination programme for Newcastle disease was introduced in 1997 due to the outbreaks of Newcastle disease in Northern Ireland. The Commission Decision of 4th. April '97 (97/262/EC) suspended Ireland's status as a non-vaccinating country.

NEWCASTLE DISEASE: SITUATION IN GREAT BRITAIN 1996 - 1997

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Investigations of Newcastle disease in poultry during 1996-97.
During 1996 a total of 44 suspected cases of Newcastle disease (ND) was investigated in poultry. Viruses were isolated from three of the investigations (Table 1). Two of these were clearly identified as vaccine viruses, one from turkeys with an intracerebral pathogenicity index [ICPI] of 0.23 identified as Hitchner B1 by monoclonal antibody binding tests, and the other from broilers with an ICPI 0.06 identified as La Sota (Clone 30). The third virus isolated from pheasants was identified as the pigeon variant NDV [PPMV-1] and ICPI values in the range 0.9-1.19 were obtained [1]. Newcastle disease was formally confirmed in this flock of pheasants and the statutory requirements implemented. This was the first outbreak of ND in Great Britain since 1984.

Table 1. Newcastle disease investigations in poultry during 1992-1997.

	1992	1993	1994	1995	1996	1997
Cases investigated	17	16	22	11	44	107
NDV isolated	1	4	11	2	3	23
Confirmed cases	0	0	0	0	1	11

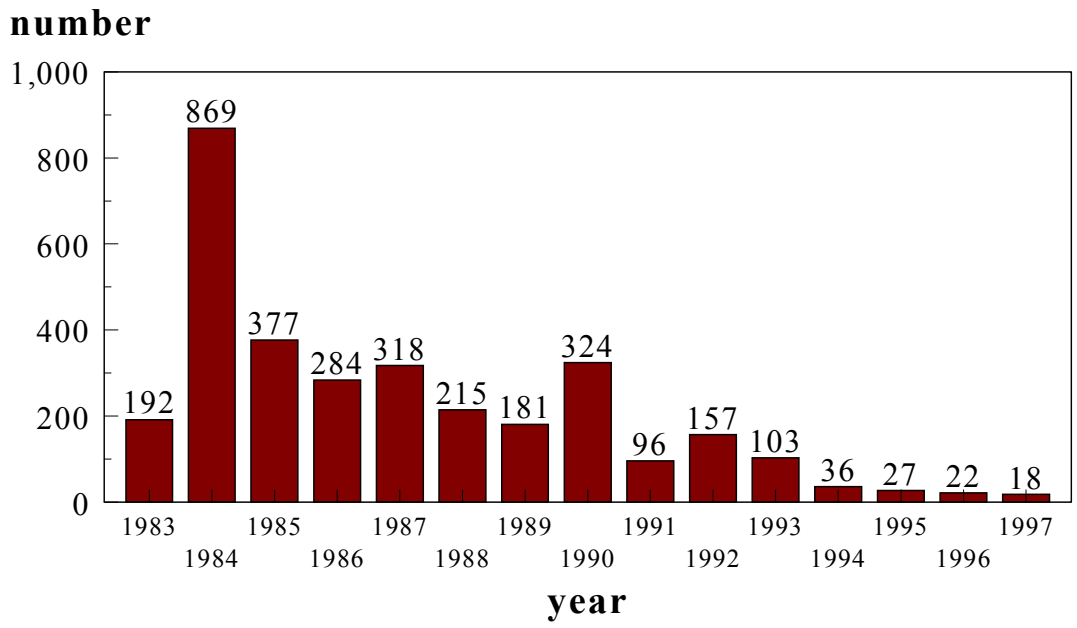
The situation in 1997 was quite different [2]. A total of 107 investigations resulted in the confirmation of 11 outbreaks of Newcastle disease between 06.01.97 and 23.04. 97, four in broiler chickens and seven in turkeys. Although the viruses isolated from these outbreaks gave ICPI values between 1.65 to 1.95, the clinical disease signs seen in field infections were variable and not always associated with high mortality, especially in turkeys. Epidemiological investigations indicated that the majority of the outbreaks occurred as a result of secondary spread by human agency from two or more primary infected flocks. The presence of similar outbreaks in Scandinavian countries in 1996 and the unusual patterns of movement of migratory birds at the end of 1996 and beginning of 1997 suggest they may have been responsible for the primary introduction of the causative virus into Great

Britain. Twelve other isolates of NDV were obtained during the investigations, each of these proved to be vaccine virus either NDW, Hitchner B1 or La Sota (clone 30) by monoclonal antibody binding and ICPI tests.

ND in racing pigeons

Infections of racing pigeons with the variant PPMV-1 virus may be confirmed in Great Britain by clinical signs alone, serology, virus isolation or a combination of these. The numbers of confirmed cases in Great Britain since the introduction of this variant virus in 1983 are shown in Figure 1. The number of confirmed outbreaks each year has continued to decline since the enforcement of the requirement to vaccinate birds taking part in races.

Figure 1. Confirmed outbreaks of PPMV-1 infections in racing pigeons in Great Britain



ND in other birds

In March and again in April 1997 birds in two quarantine premises were confirmed as infected with ND viruses. Chronologically, these quarantine outbreaks occurred between the 8th and 9th poultry outbreaks and both premises were also geographically close to the affected poultry premises. ND viruses isolated had ICPI values of 1.65 and 1.81. The mAb-binding pattern of one of the quarantine NDV isolates was identical to the virus responsible for the poultry outbreaks, but the other showed differences in the binding of two of the 26 mAbs. The two quarantine isolates were shown by nucleotide sequencing and phylogenetic analysis to be

genetically distinct both from the isolates from the Great Britain poultry outbreaks and each other.

References

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2. Alexander, D.J., Morris, H.T., Pollitt W.J., Sharpe C.E., Eckford R.L., Sainsbury R.M.Q., Mansley L.M., Gough R.E., Parsons G. (1998) Newcastle disease outbreaks in domestic fowl and turkeys in Great Britain during 1997. Veterinary Record 143, 209-212.

COUNTRY REPORT - NEWCASTLE DISEASE IN NORTHERN IRELAND.

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1. The August 1996 Outbreak.

An incident involving pigeon PMV-1 infection of a commercial layer flock in County Antrim occurred in August 1996. No clinical signs were seen with the exception of the production of a small number of pale-shelled eggs each day. An isolate was made from tracheal samples submitted. The isolate had an ICPI of 1.65, defining it as Newcastle disease virus and resulted in the flock being slaughtered. Protection and surveillance zones were imposed around the affected premises. The isolate was further identified as being the pigeon variant of PMV-1 (PPMV-1) by monoclonal antibody binding.

Epidemiological investigation of the outbreak concluded that the most likely source of infection was through contact with wild birds/pigeons.

2. The February 1997 Outbreak.

(a). Summary.

With the exception of BSE, the epizootic of Newcastle disease which occurred in early 1997 was the most significant disease episode to affect the Northern Ireland agri-food industry in recent times.

Over a period of 11 weeks, the disease affected a total of 26 locations, spread over each of the major industry sectors and organisations. Outbreaks occurred in every county except Fermanagh and involved the loss of 1.3M poultry in total. A single further isolated incident of unknown origin occurred in mid August 1997. It involved a small game farm on the shores of Lough Neagh.

This disease was brought under control by a combination of slaughtering-out and disposal, cleaning and disinfection, movement controls, and vaccination.

On Sunday February 2nd 1997, notification was received of high mortality in a broiler flock near Lurgan. Velogenic Newcastle Disease was confirmed later that week and all birds on the premises were slaughtered. One week later another broiler flock was reported and subsequently confirmed; investigations linked it as secondary to the Lurgan outbreak.

Over the next 2 weeks, 8 further outbreaks were confirmed, including flocks associated with 4 major poultry organisations and all the major feedmills in the Province. With the exception of 2, all outbreaks were located within 4 km of major bodies of water (Lough Neagh, Strangford and Larne Loughs). The source of the epidemic was unclear although transmission from wild birds was suspected given the proximity of the premises to water and identical virus being linked to wild birds in other European countries.

A further 13 outbreaks were declared during the following 8 weeks, affecting all types and ages of poultry. Epidemiological investigation of these outbreaks revealed that the major factors involved in rapid spread of the disease were as follows:

(i) Mechanical spread via contaminated equipment e.g. egg trolleys, crates.

(ii) Mechanical spread via personnel. In 3 outbreaks at least, the most likely source was staff moving from an infected flock to non-infected premises. This may have happened before disease was noticed on the infected premises. Indirect contact through advisors in one organisation is thought to have played a major role in rapid dissemination of infection.

(iii) Airborne spread. Epidemiological investigation found wind-borne virus to be the most likely cause of the rapid northward spread of the epidemic after it was established in a large flock in County Antrim.

(iv) Mechanical spread from a hatchery. A final group of 3 reported outbreaks were attributed to contamination of day-old chicks immediately after removal from the hatchers.

In mid August 1997, a single isolated incident of disease was confirmed in a small group of un-vaccinated free-range pheasants being reared for game. Despite intensive investigations it has so far proven impossible to determine a specific source for this outbreak.

b) Vaccination.

Prior to the outbreak, Northern Ireland had maintained a policy of non-vaccination for Newcastle disease. While compulsory vaccination was required within protection and surveillance zones from the outset, voluntary vaccination was permitted throughout the remainder of the province from February 17th. Compulsory vaccination was introduced within a defined area on March 10th and subsequently extended to cover all of Northern Ireland on April 3rd.

c) Laboratory investigations.

Diagnostic procedures for the confirmation and differential diagnosis were carried out as described in Annex III of Directive 92/66, while the testing of poultry prior to slaughter from within surveillance zones was carried out according to the Annex of Decision 95/117.

Intracerebral pathogenicity indices (ICPIs) were determined for all isolates and the values ranged from 1.62 to 1.94. The isolates were also submitted to VLA Weybridge for monoclonal antibody typing and were all found to be Group C1 avian paramyxovirus type 1.

While the scale of the laboratory work required for confirmation and differential diagnosis of ND in a major epizootic had been anticipated, and contingency plans prepared, the additional work concerned with trade guarantees had been underestimated. It is useful to provide some data on the numbers of laboratory assays required in this outbreak, in relation to the size and nature of the poultry industry in Northern Ireland.

The total population is 14 million birds. There are 240 broiler units and 150 layer units of >1000 birds. 48 farms have greater than 50,000 birds. The broiler sector is dominated by two large integrated organisations, while the layer farms are largely independent, though with links to common feed suppliers and packing stations.

Serology was useful as a diagnostic method in the early stages of the outbreak, but of lesser value once vaccination became compulsory. Nevertheless, an additional 16,000 HI tests were carried out as a consequence of the epizootic. The majority of these tests were undertaken to monitor the efficacy of vaccination, though titres, particularly in broilers at slaughter, were monitored for evidence of infection, i.e. titres higher than those expected as a result of vaccination.

Diagnosis and epidemiological investigation required that 1,400 samples be processed for attempted virus isolation, while the additional work to meet the trade guarantee requirements involved a further 51,000 samples (850 flocks).

One of the major problems arising from this work was the limited supply of suitable embryonated eggs for use in virus isolation.

EUROPEAN COMMISSION
DIRECTORATE-GENERAL VI
AGRICULTURE
Public, animal and plant health
VI.B.II.2 Veterinary and zootechnical legislation

EU COMMISSION REPORTS FOR 1996 - 1997

MEASURES TO CONTROL AVIAN INFLUENZA AND NEWCASTLE DISEASE IN THE EUROPEAN COMMUNITY

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Introduction

Avian influenza (AI) and Newcastle disease (ND) are viral diseases of poultry and wild birds. Both diseases are classified as "List A" diseases by the International Animal Health Code of the Office International des Epizooties (OIE). This classification means that the diseases:

- have the potential for very serious and rapid spread, irrespective of national borders,
- are of serious socio-economic importance,
- are of major importance in the international trade of live poultry, poultry meat, eggs and other products originating from poultry.

The purpose of this paper is briefly to review the veterinary policies applied by the EC to the control and eradication of Avian Influenza and Newcastle disease and to review the disease situation.

Control and eradication of AI and ND

a) Control measures

The measures adopted to control and eradicate AI and ND are based on the strategy of stamping-out infected flocks and controlling the movement of poultry, products originating from poultry, vehicles and any other substance liable to transmit virus. To ensure such actions in the event of an outbreak, Member States have obligations:

- to arrange for an investigation to confirm or rule out the presence of disease when poultry are suspected of being infected,
- to place holdings under surveillance and prohibit movements to and from holdings during the surveillance period, when disease is suspected,
- to apply stamping-out when disease has been confirmed on a holding,
- to perform a thorough epidemiological inquiry when disease is suspected and confirmed,
- to establish protection zones and surveillance zones around infected holdings.

In addition to the obligations listed above, the legislation on the control of AI and ND include requirements for:

- designation of national laboratories and a Community reference laboratory,
- control measures to be applied when swill is fed to poultry,

- a contingency plan. Each Member State shall present a contingency plan for approval by the Commission. The plans must contain provisions to supply the necessary equipment, facilities and expert staff to deal with an epidemic of a reasonable size.

b) Competence for control measures

The responsibility for the implementation of control measures rests with the Member States. The Commission is responsible for ensuring that measures are fully and properly applied.

The epizootic disease situation within the Community is normally reviewed once a month by the Standing Veterinary Committee. The Commission may ask the Committee to give its opinion on proposals for extra disease protection measures, if the Commission considers that the measures taken by the national authorities are not adequate. When such protection measures are introduced the principle of regionalisation is usually applied and the measures are adopted within the framework of Council Directive 90/425/EEC concerning Veterinary and Zootechnical checks applicable in intra-Community trade.

c) The regionalisation policy

Regionalisation is the application of measures to control and eliminate animal disease from an infected area. It replaces the old policy of applying measures at the borders of the affected country, a policy which is not compatible with the Single Market. The concept of regionalisation has been accepted as the basis for international policy by OIE and many trading partners. More recently Regionalisation has been included in the Agreement on the application of Sanitary and Phytosanitary measures adopted by the World Trade Organisation (WTO). It should restrict trade from the designated region but permit trade from the unaffected part of the country without risk to other Member States.

To facilitate a decision to regionalize part of a Member State as distinct from a decision to block an entire Member State, a number of conditions should be met. These include:

- A detailed epidemiological enquiry must have been carried out which has resulted in sufficient information to enable the geographic limits of the region to be clearly defined.
- Restrictions on movements out of the Region must apply to all other areas.
- The boundary of the region must be easily controlled.
- Police controls must be in place to prevent all prohibited movements.
- Eradication measures must be such as to allow the disease to be eradicated in a limited period.
- A single crisis unit with all the necessary powers must be in charge of the eradication campaign.

The use of regionalisation in relation to disease control and trade has been demonstrated to be beneficial both for Member States affected by "List A" diseases and those unaffected.

d) Financial support and compensation

The Council, by Decision 90/424/EEC, established a fund for veterinary expenditure. In accordance with the provisions of this decision Member States can obtain a financial contribution from the Community towards the eradication of AI and ND. The level of compensation is normally up to 50% of Member States' costs, which relate to slaughter of animals, cleaning and disinfection and destruction of contaminated materials. Within the framework of the same Decision financial contribution can be made available to cover expenditure on national disease programmes, operation of disease reference laboratories and strengthening veterinary infrastructures. The Commission has during 1996 and 1997 adopted several Decisions concerning Community financial assistance related to the control of ND.

DISEASE SITUATION

Avian influenza and Newcastle disease are reported by Member States in accordance with the provisions of Council Directive 82/894/EEC. During the period 1994-1997 outbreaks have been reported as shown in Tables 1 and 2.

Table 1: Number of outbreaks of avian influenza reported by Member States

COUNTRY	1994	1995	1996	1997
Austria	0	0	0	0
Belgium	1	0	0	0
Denmark	0	0	0	0
Finland	0	0	0	0
France	0	0	0	0
Germany	0	0	0	0
Greece	0	0	0	0
Spain	0	0	0	0
Ireland	0	0	0	0
Italy	0	0	0	7
Luxembourg	0	0	0	0
Netherlands	0	0	0	0
Portugal	0	0	0	0
Sweden	0	0	0	0
United Kingdom	0	0	0	0
TOTAL	1	0	0	7

Table 2: Number of outbreaks of Newcastle disease reported by Member States

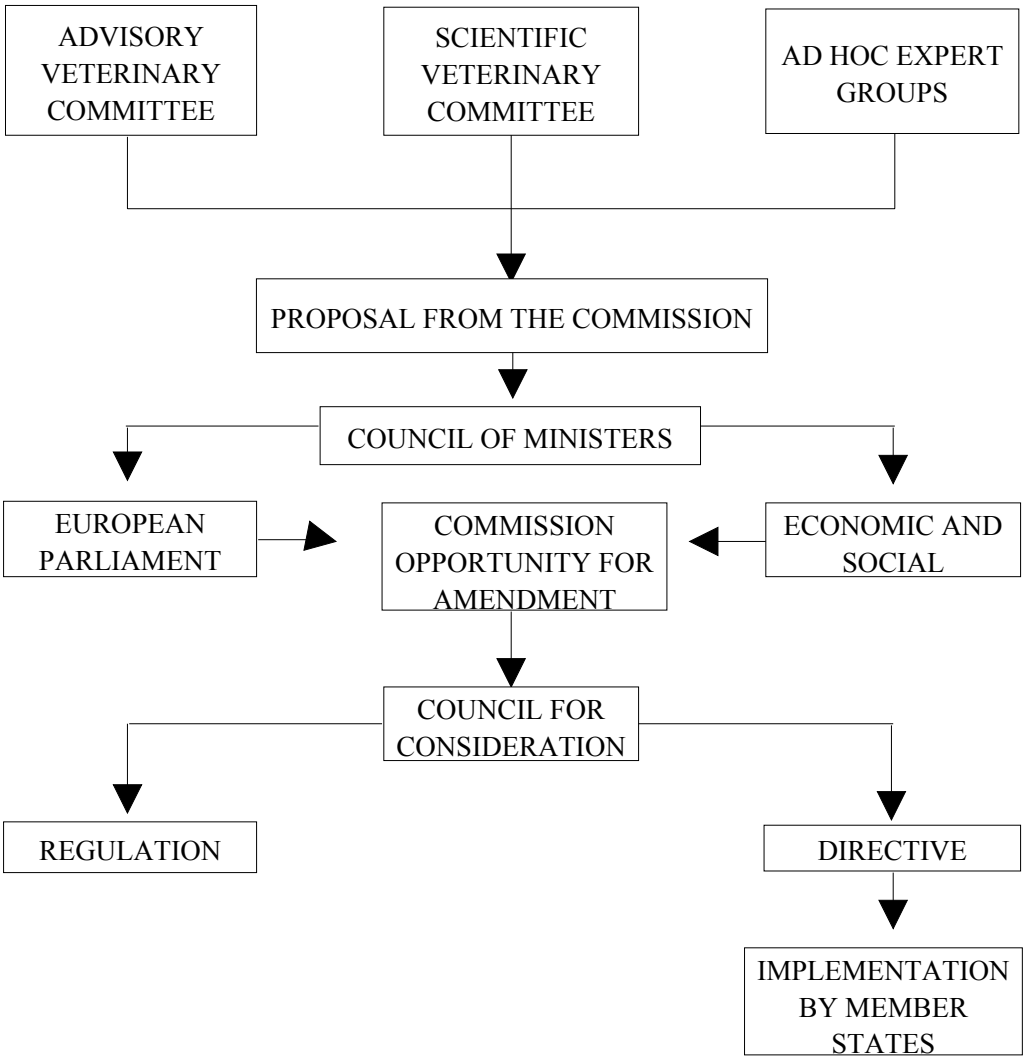
COUNTRY	1994	1995	1996	1997
Austria	0	0	4	2
Belgium	1	11	7	2
Denmark	0	14	4	0
Finland	0	0	2	0
France	0	0	0	1
Germany	173	28	2	0
Greece	0	0	0	0
Ireland	0	0	0	1
Italy	42	2	4	2
Luxembourg	0	2	0	0
Netherlands	8	5	2	1
Portugal	12	2	3	10
Spain	13	0	0	0
Sweden	0	1	0	1
United Kingdom	0	0	2	38
TOTAL	236	65	30	58

GENERAL LEGISLATIVE MATTERS

During the development of animal health legislation within the European Union the Commission will draw upon the expertise present in all Member States. This includes assistance from the Scientific Veterinary Committee where the members are nominated from highly qualified scientific persons having competence in the area of animal health. The Advisory Veterinary Committee where members are representing agricultural producers, agricultural cooperatives, industry, commerce, workers, consumers and the Federation of European Veterinarians; ad hoc working groups where the participants are coming from National Veterinary Administrations and Laboratory Services of Member States. Furthermore, input may be obtained from international organisations such as the International Office of Epizootics (OIE), the European Pharmacopoeia and the European Federation of Animal Health (FEDESA). Following the completion of the technical work a draft proposal will be adopted by the Commission and it will be sent to the Council of Ministers for adoption as a legal instrument via the Community's decision-making procedures. At present there are six different procedures available for adoption of legislation involving the European Parliament, the Council and the Commission.

The consultation procedure which has frequently been used, will be described (Fig.1). A proposal is sent from the Commission to the Council. The Council requests an opinion from the European Parliament and, in most cases, from the Economic and Social Committee. Once these opinions have been given, the Commission has the opportunity to amend the proposal if it so wishes. The proposal is then examined by the Council which may adopt it as proposed, adopt it in an amended form, or fail to reach agreement, in which case the proposal remains «on the table». The text of all proposals and adopted legislation is published in the Official Journal of the European Communities.

Figure 1. Development and adoption of legislation. The consultation procedure



ANIMAL HEALTH CONDITIONS FOR TRADE WITHIN THE EU

a) Trade in poultry and hatching eggs

The general animal health requirements applicable to trade poultry pigs are laid down in Council Directive 90/539/EEC, whilst the requirements to veterinary checks to be carried out at the place of origin (dispatch) are given in Council Directive 90/425/EEC. Both Directives refer to the use of animal health certificates. In general terms an animal health certificate is a document which contains a statement made by an official veterinarian about the condition of the animal or animals covered by the certificate. This makes the Animal Health Certificate a cornerstone of the legislation in relation to trade within the Community and puts requirements on the veterinarian signing the certificate and on the veterinary administration in the Member State of origin.

b) Trade in fresh poultry meat

The general rules governing intra-community trade in poultry meat are given in Council Directive 91/494/EEC. Meat is in the said Directive defined as any parts of poultry which are fit for human consumption.

ANIMAL HEALTH CONDITIONS FOR IMPORTS FROM THIRD COUNTRIES

a) Imports of live poultry and hatching eggs.

The general conditions for importation from third countries are laid down in Directive 90/539/EEC. A main requirement is given in Article 22 shown below:

"Article 22

1. Poultry and hatching eggs must come from third countries:

   a) in which Avian Influenza and Newcastle disease, as defined in Council Directives 92/40/EEC and 92/66/EEC respectively, are legally notifiable diseases;

   b) free from Avian Influenza and Newcastle disease,

      or

      which, although they are not free from these diseases, apply measures to control them which are at least equivalent to those laid down in Directives 92/40/EEC and 92/66/EEC respectively.

2. Additional criteria for classifying third countries in respect of paragraph 1(b), particularly as regards the type of vaccine used, shall be adopted in accordance with the procedure laid down in Article 32 before 1 January 1995.

3. The Commission may, in accordance with the procedure laid down in Article 32, decide under which conditions paragraph 1 is to apply only to a part of the territory of third countries."

   The Commission has in accordance with the provisions of Article 22(2) of Directive 90/539/EEC by Commission Decision 93/342/EEC laid down the criteria for classifying third countries with regard to Avian Influenza and Newcastle disease in relation to imports of live poultry and hatching eggs.

b) Imports of poultry meat

The basic animal health conditions for importation of poultry meat from third countries are laid down in Directive 91/494/EEC. A main requirement is given in Article 10 shown below:

"Article 10

1. Fresh poultry meat must come from countries:

   a) in which Avian Influenza and Newcastle disease are legally notifiable diseases throughout the country in accordance with international standard;

   b) free from Avian Influenza and Newcastle disease,

      or

      which, although they are not free from these diseases, apply measures to control them which are at least equivalent to those laid down in Directives 92/40/EEC and 92/66/EEC respectively.

2. Additional criteria for classifying third countries in respect of paragraph 1 shall be adopted in accordance with the procedure laid down in Article 17 before 1 January 1995.

   When implementing paragraph 1, the Commission shall adopt, by means of certification, all measures necessary to safeguard the particular animal health situations in certain regions of the Community.

3. The Commission may, in accordance with the procedure laid down in Article 17, decide under which conditions paragraph 1 is to apply only to a part of the territory of third countries."

   The Commission has in accordance with the provisions of Article 10(2) of Directive 91/494/EEC by Commission Decision 94/438/EEC laid down the criteria classifying third countries and parts thereof with regard

to Avian Influenza and Newcastle disease in relation to imports of fresh poultry meat.

REVIEW OF THE DEFINITION OF NEWCASTLE DISEASE

In the report of the meeting of the OIE standards Commission (Paris, 24-26th September 1997) it is stated concerning the definition of Newcastle disease:

Newcastle disease

Experts in the OIE Reference Laboratories for Newcastle disease had advised the Standards Commission that the current OIE definition of this disease was rather imprecise and could lead to confusion in its interpretation. The Commission proposed the following definition, which was agreed by the Code Commission and will be submitted for the approval of the International Committee in May 1998:

> "Newcastle disease is a disease of poultry caused by a virus of avian paramyxovirus serotype 1 (APMV-1) which has an intracerebral pathogenicity index (ICPI) in 1-day-old chicks of 1.2 or greater."
>
> "Virulent virus can also be confirmed by the presence of multiple basic amino acids at the C-terminus of the F2 protein and F (phenylalanine) at residue 117, the N-terminus of the F1 protein; failure to demonstrate this amino acid sequence would require characterisation by ICPI test."
>
> The definition will include both velogenic and mesogenic strains of the virus, including certain vaccine strains. It excludes viruses from wild birds. The inclusion of molecular typing as an alternative to ICPI should reduce the need for inoculation of 1-day-old chicks. This would be beneficial both on welfare grounds and in reducing the problem, encountered by some laboratories, of having a constantly available supply of such birds."

The European Commission is at present studying the proposal from OIE in co-operation with Member States. During this study it is taking into account the definition adopted by the European Union in Council Directive 92/66/EEC. In the said Directive "Newcastle disease" means an infection of poultry caused by any avian strain of the paramyxovirus 1 with an intracerebral pathogenicity index (ICPI) in day-old chicks greater than 0.7.

REPORT OF THE EUROPEAN UNION REFERENCE LABORATORIES FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE 1996-1997

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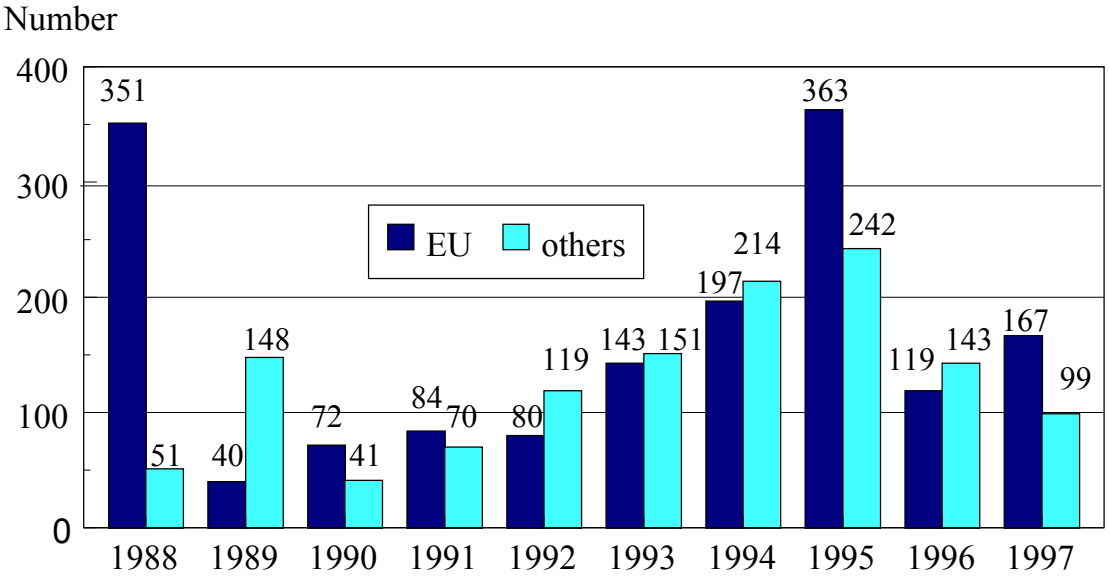
Viruses received by International Reference Laboratory

The numbers of viruses submitted by all countries to the reference laboratory for each of the years 1988 to 1997 are shown in Figure 1. The number of viruses submitted in a particular year does not necessarily mean that the viruses were isolated that year and the reference laboratory is often asked to characterise isolates retrospectively.

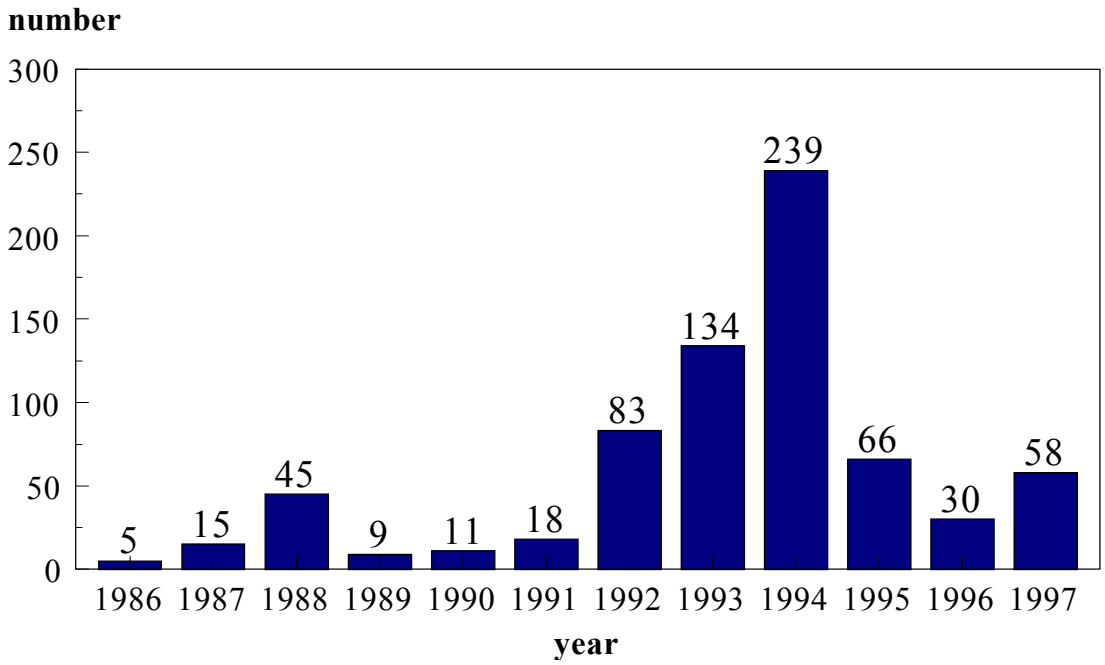
Figure 1 Viruses submitted to the International Reference Laboratory CVL Weybridge during 1988 to 1997



The numbers of viruses received in 1996 and 1997, 262 and 266 respectively, represent a decline from the peak of 605 in 1995. While this is in keeping with the peaking of ND outbreaks in EU countries in 1994 (Figure 2), with a rise in EU submissions in 1997 due the outbreaks in the United Kingdom, there is no explanation of why the submissions from non-EU countries show a similar pattern.

The possibility that submissions and the outbreaks in EU countries represent the world wide spread of ND during those years seems unlikely as the conclusion from characterisation of the viruses isolated was that both temporally and geographically different viruses were involved.

Figure 2. Reported outbreaks of Newcastle disease in EU countries 1986-1997.



Influenza viruses submitted during 1996-1997

Influenza virus submissions are listed in Table 1. Submissions from non-EU countries were restricted to two viruses from ostriches in Zimbabwe in 1996 and six viruses isolated in Singapore as a result of virological monitoring of birds imported into Singapore, mainly from other Asian countries. Isolates received from EU countries were usually the result of testing imported birds or the occasional infection of domestic poultry. The exception being the viruses submitted from the outbreaks of highly pathogenic avian influenza in mixed poultry flocks in Italy in 1997 (Fioretti et al, 1998) which were identified as H5N2 viruses with extremely high IVPI values.

Isolates made from ostriches in The Netherlands and Denmark were H5N2 viruses of low virulence for chickens as was one of the viruses from ostriches submitted from Zimbabwe.

Submissions of viruses of H9N2 subtype are in keeping with the apparent prevalence of this subtype in different types of birds throughout the world, which appears to have gradually increased following reports of virus of this subtype

combination, or of H9 viruses with a different neuraminidase, in poultry in several Asian countries from 1994.

Table 1: Influenza A viruses submitted to the International Reference Laboratory during 1996 and 1997*.

Country	Number	Host	Subtypes	IVPI
1996				
		non-EU countries		
Zimbabwe	1	ostrich	H5N2	0
Zimbabwe	1	ostrich	H7N1	0
Total	**2**			
		EU countries		
Italy	1	pig	H3N2	0
Italy	2	chicken	H3N2	0
Italy-Q†	1	psittacines	H4N6	0
Great Britain	1	mallard	H4N6	0
Netherlands (Q)	2	ostriches	H5N2	0
Denmark (Q)	14	ostriches	H5N2	0 (3 tested)
Great Britain	1	human	H7N7	0
Italy	1	chicken	H9N2	nd
Germany	8	turkeys	H9N2	0
Germany	1	chicken	H9N2	nd
Germany	1	duck	H9N2	nd
Total	**31**			
1997				
		non-EU countries		
Singapore-Q	2	caged birds	H3N6	0 & 0.8
Singapore-Q	1	caged birds	H4N6	0.45
Singapore-Q	1	duck	H5N3	0
Singapore-Q	2	duck	H9N2	0
Total	**6**			
		EU countries		
Austria	2	pigs	H1N1	nd
Great Britain-Q	9	caged birds	H4N6	0.4-0.7
Italy	**7**	**poultry**	**H5N2**	**2.9-3.0**
Ireland	1	pheasants	H9N2	0
Total	**19**			

**Not all viruses received had been isolated within this period.*
†*Q = imported birds held in quarantine.*

Nucleotide sequencing H5 and H7 viruses.
Following RT-PCR and nucleotide sequencing the amino acid sequence at the cleavage site of the haemagglutinin precursor protein of each H5 and H7 virus submitted was deduced. In keeping with the in vivo virulence tests only the Italian H5N2 isolates had multiple basic amino acids at the cleavage site of the HA0 protein (Table 2).

Table 2. Deduced amino acid sequences at the HA0 cleavage site of H5 and H7 viruses submitted to the International Reference Laboratory.

Virus	IVPI	amino acid sequence
ostrich/NL-Q/110/96 (H5N2)	0.00	-PQRETR↓GLF-
ostrich/DK-Q/418/96 (H5N2)	0.00	-PQRETR↓GLF-
ostrich/DK-Q/725/96 (H5N2)	0.00	-PQRETR↓GLF-
Zimbabwe 1996 (H5N2)	0.00	-PQRETR↓GLF-
Zimbabwe 1996 (H7N1)	0.00	-PEIPKGR↓GLF
poultry/Italy/97 (H5N2)	**3.00**	**-PQRRRKKR↓GLF-**
duck/Singapore-Q/97 (H5N3)	0.00	-PQRETR↓GLF-

Paramyxoviruses received during 1996
The viruses received from EU countries in 1996 (Table 3) were consistent with the disease situations reported.

Table 3: European Union countries submitting PMV-1 viruses during 1996*.

Country	PMV-1	PPMV-1	PMV-3	PMV-4
Great Britain	2	13		
N. Ireland	2	2		
Germany	13		1	
Ireland	5	7		
Austria	1	3		
Portugal	14	1		
Italy	3	5		
Sweden			1	1
France	1			
Finland	1	3		
Denmark	9			
Total	**51**	**35**	**2**	**1**

**Not all viruses received had been isolated in 1996 and numbers may include viruses isolated in countries other than the submitting country.*

There was an overall decline in EU submissions in 1996, especially from Germany where measures seemed to have brought the outbreaks seen in backyard flocks in 1994-1995 under control. The number of PPMV-1 viruses submitted was about the same as in 1995.

In 1996 a total of 115 viruses typed as PMV-1 viruses (Table 4), was received from 12 countries from outside the EU representing a wide cross section from different continents and geographical areas.

Table 4: Other countries submitting PMV-1 viruses during 1996*.

Country	PMV-1	not viable
India	26	12
South Africa	4	2
U.A.E.	57	12
Iran	2	
Switzerland	2	
Mexico	1	
Oman	2	
Morocco	6	
Bolivia	1	
Czech Republic	4	
Poland	9	
Canada	1	
Total	**115**	**26**

**Not all viruses received had been isolated in 1996.*

Paramyxoviruses received during 1997

There was an increase in PMV-1 virus submissions from EU countries in 1997 (Table 5). The majority of these were as a result of the outbreaks occurring in the United Kingdom in that year and were increased by multiple isolations from outbreaks, the isolation of vaccine viruses from investigations of disease and from sampling for export purposes. Some of the viruses submitted from Germany were isolates made in non-EU countries.

One notable change from previous years was the low number of PPMV-1 viruses submitted from EU countries in 1997, only 5 compared with 35 in 1996. However, the number of such viruses submitted each year does tend to fluctuate widely. The reasons for this are unclear, but hopefully this time it may be indicative of the gradual decline and disappearance of infections of pigeons with this variant PMV-1 virus, which was first recorded in the EU some 17 years ago.

Table 5: European Union countries submitting PMV-1 viruses during 1997*.

Country	PMV-1	PPMV-1	PMV-2
Great Britain	47	1	
N. Ireland	24		
Germany	41	1	
Ireland	4		
Portugal	13		
Italy		2	
Sweden	1		
Netherlands	3		
Finland	1		
Austria		1	
Belgium			1
Denmark	6		
Norway	1		
Total	**142**	**5**	**1**

**Not all viruses received had been isolated in 1997 and numbers may include viruses isolated in countries other than the submitting country.*

PMV-1 submissions from non-EU countries in 1997 (Table 6) showed a marked decline and of the 73 viruses received 67, from UAE and Taiwan, were associated with specific retrospective surveys and did not reflect the current situation in those countries.

Table 6: Other countries submitting PMV-1 viruses during 1997*.

Country	PMV-1	not viable
Saudi Arabia		18
South Africa	1	
U.A.E.	32	1
Switzerland	2	
USA	1	
Singapore	2	
Israel		1
Taiwan	35	
Total	**73**	**20**

**Not all viruses received had been isolated in 1997.*

Antigenic grouping of Newcastle disease virus [PMV-1] isolates.
All PMV-1 viruses received during 1996 to 1997 were characterised by their ability to cause binding of a panel of 28 monoclonal antibodies [mAbs] to cell cultures infected with the viruses. For convenience and the need to summarise, the results are shown in mAb groups (Alexander et al, 1997) in Tables 7-10. Generally, viruses in the same group show identical binding patterns for all 28 mAbs used. However, some isolates in the same group may vary by their ability to react with one or two mAbs.

All viruses placed in the same group tended to share the same biological properties and for convenience the groups have been separated in Tables 6-9 on the basis of virulence for chickens.

Table 7. Antigenic grouping of Newcastle disease virus isolates from EU countries submitted during 1996

Country	Number of viruses in monoclonal antibody binding group										
	velogenic						*lentogenic*				
	P	**B**	**C1**	**DE**	**?**	**Port**	**C2**	**E**	**G**	**H**	**L**
GB	13							2			
Germany	1	1		9				2			
Finland	3		1								
Italy	5				2			1			
Portugal	1					14		2			
Ireland	7									1	4
N. Ireland	2										2
Denmark			7				1		1		
Austria	3	1									

In both years and from EU and non-EU countries the predominant mAb groups of virulent isolates were C1 and P. This reflects the panzootic nature of viruses showing these binding patterns. Viruses received from Taiwan represented viruses isolated between 1969-1995. It was of interest that some of the earliest isolates showed pattern C1 as although that pattern was produced by chicken/Kuwait/68 the nearest known C1 isolate after that had been in 1977.

The majority of viruses of low virulence received in both years and from all sources were placed in mAb groups consistent with the live vaccines used or otherwise present in the country of origin i.e. group E - B1/La Sota; group G - NDW; group F - strain F and group D - Komarov/Mukteswar. Other lentogenic virus mAb groups were those usually associated with viruses isolated from feral birds.

Table 8. Antigenic grouping of Newcastle disease virus isolates from other countries submitted during 1996.

Country	Number of isolates in monoclonal antibody binding group								
	velogenic						*lentogenic*		
	P	**B**	**C1**	**A**	**?[a]**	**J**	**F**	**E**	**D**
UAE	7	8	24	7	15	3			
S. Africa			2		2				1
Bolivia								1	
India			1		8		1	14	2
Iran			2						
Switzerland	1							1	
Oman				2					
Morocco					6				
Czech Rep.			2		2				
Poland								8	1
Canada					1				

[a]*not grouped - viruses placed in this category did not necessarily show the same mAb pattern.*

Table 9. Antigenic grouping of Newcastle disease virus isolates from EU countries submitted during 1997

Country	Number of viruses in monoclonal antibody binding group								
	velogenic						*lentogenic*		
	P	**B**	**C1**	**DE**	**?**	**Port**	**E**	**G**	**H**
GB	1		25				6	16	
Germany[†]	1			1			15		
Finland									1
Italy	2								
Portugal						12	1*		
Ireland			1				3		
N. Ireland			13		6		5		
Denmark							5		1
Austria	1								
Netherlands			1		2				
Belgium		1							
Sweden			1						
Norway			1						

[†]25 viruses not done, *******IVPI 1.55!*

Table 10. Antigenic grouping of Newcastle disease virus isolates from other countries submitted during 1997

Country	Number of isolates in monoclonal antibody binding group						
	velogenic				*lentogenic*		
	P	**C1**	**?[a]**	**J**	**E**	**G**	**H**
UAE	8	6	15	3			
S. Africa		1					
Switzerland					2		
USA			1				
Singapore			1			1	
Taiwan	1	24	6		1	1	2

[a]*not grouped - viruses placed in this category did not necessarily share the same mAb pattern.*

Acknowledgements
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NEWCASTLE DISEASE IN OSTRICHES IN SOUTHERN AFRICA

NEWCASTLE DISEASE IN SOUTH AFRICA: CONTROL AND RESEARCH IN OSTRICHES

D.J. Verwoerd, C. Brückner, W.P. Burger and M.E. Mogajane

NEWCASTLE DISEASE IN SOUTH AFRICA: AN OVERVIEW OF EXISTING AND PROPOSED CONTROL MEASURES

A Introduction

Newcastle Disease Virus (NDV)) is the most important infectious agent influencing poultry production throughout the world, both in the commercial sector as well as in subsistence farmer ("village chickens") level. Most strains are spread in intensive poultry houses by aerosol or by the faecal-oral route, while feed trucks, personnel, etc. can act as mechanical carriers spreading the virus to other areas.

Newcastle disease was first diagnosed in South Africa in 1944. A slaughter out policy was followed until 1967. In 1971 a vaccination policy was introduced and since then only isolated outbreaks have occurred. South Africa experienced a particularly severe NDV epidemic in 1993/94 with high mortalities in poultry as well as ostriches and other avian species. NDV had previously been recorded in commercial ostriches in Israel as well as in European zoos. Isolated NDV-outbreaks occur sporadically in commercial ostriches in South Africa in most cases related to concurrent poultry (commercial and/or village, chickens) outbreaks. South African export slaughter ostriches are vaccinated against NDV with only La Sota type vaccines at least 30 days but not longer than 6 months before slaughter under supervision of a Veterinary Official. The spread of NDV through a flock of ostriches is extremely slow compared to that in commercial poultry probably due to the extensive nature of ostrich farming in South Africa.

B. Farming systems in South Africa

The poultry industry in South Africa is highly commercialised and is using an intensive fanning system. In addition to this there are village chickens which are kept in cages or free ranging for egg and meat production for the family. The ostrich industry is well organised and most ostriches are kept on extensive farms larger than 100 hectares. Majority of ostrich fanning takes place in arid areas not suitable for intensive poultry production.

Ostrich farming with domesticated birds South Africa and elsewhere started in 1863. From 1870 ostrich fanning became an extremely profitable industry, aided in no small way in 1869 by the ingenious invention of an incubator for ostrich-eggs by Arthur Douglas, the pioneer ostrich farmer of Grahamstown. Between 1900 and 1914 the industry reached a zenith in what is today known as the second ostrich-feather boom period. In 1913 ostrich-plumes ranked fourth on the list of South African exports, after gold, diamonds and wool. At this time there were already more than 77 600 ostriches on farms in South

Africa, bringing in exports earnings of close to £3000 000 through the sale of *450* 000 kg of plumes.

The founding of the Klein Karoo Agricultural Co-operative in 1945 rehabilitated the ostrich industry as a whole. In 1963/4 the world's only ostrich abattoir was erected, initially only for the production of "biltong " (dried meat) and fresh meat for local consumption. In 1969/70 a leather tannery was added and marketing of leather processed from ostrich skin started.
In 1974 the tannery was doubled in size, and a new abattoir to cope with the International demand for ostrich meat was built in 1980/81. This abattoir currently slaughters an average of 1000 birds a day. Currently there are 6 abattoirs exporting to member states. *No outbreaks of NDV has ever been associated with the export of ostrich meat anywhere in the world.*

C. Negotiations with the EU

South Africa and the European Union have been negotiating on Newcastle Disease and Avian influenza control measures since 1993. South Africa has been exporting high quality, safe ostrich meat to the European countries for many years, but unfortunately the conditions for exports came under review by the inclusion of poultry in the definition of poultry by article 7 of Council Directive 92/65/EEC of 13 July 1992.

Although the South African Veterinary Services and the European Commission had been negotiating intensively, no resolution has been agreed upon.

D. Control measures

Newcastle disease is a compulsory notifiable disease in South Africa according to the Animal Diseases Act, (Act 35 of 1984). Zoosanitory measures in accordance with the OIE Animal Health Code.
Quarantine measures are imposed around outbreak areas to prevent the spread of Newcastle disease and are maintained until clinical end point is reached. Movement of poultry, ostriches and other birds is forbidden. (For hatching eggs a special dispensation is granted by the Director Animal Health)
Import policy control the importation of live birds, whether poultry, ostriches, zoological parks, personally owned pets or commercial pet retail market.
Compulsory ring vaccination around outbreaks practised
Surveillance monitoring is maintained around outbreaks

1 OSTRICHES

Control measures are aimed at minimising the risk of virus transmission by controlling the disease at the holding/farm of origin, vaccination policy, tick and

rodent control program, residue protection, control during outbreaks and management practices at the abattoirs.

1.1 Holding/farm of origin

All holdings/farms supplying ostriches for export slaughter are registered with the Directorate Animal Health in order to conform to certain minimum standards.

The surrounding area, within a radius of 3km must be free of Newcastle disease, highly pathogenic Avian Influenza and Ostrich Influenza.
The slaughter birds come from a holding/farm which is not under Animal Health restrictions.
The holding/farm must conform to Minimum quarantine principles. This means there must be a perimeter fence which restrict access to the holding. Entrances to the holding/farm is controlled.

All ostriches are individually and permanently identified according to the method prescribed by the National Director Animal Health. Such identification is done on entering the holding/farm at least 3 months of age.

Detailed records are kept in the holding have details of arrival dates, origin, identification numbers, acaricide treatment and vaccination dates, rodent control program, mortalities and results thereof. All records should be available on request by the Veterinary officer.

The camps designated to hold ostriches during the final 30 days of pre-slaughter isolation are devegitated. A 3m wide strip around these camps is devegitated.

Transport through infected area is prohibited unless approved road or rail links are used.

The registration of a holding is not transferable to new management nor between holdings under control of the same management.

1.2 Vaccination

Ostriches: Vaccination is done at least 1 month but not longer than 6 months prior to slaughter. Vaccination shall consist of a lentogenic eye/nose drop vaccine plus an inactivated lentogenic indictable vaccine. Only registered lentogenic vaccines are used under supervision of a Veterinary official.

1.3 Tick and rodent control

Acaricide treatment is done between 30 and 14 days prior to slaughter. Only deltamethrin or flumenthrin camps after acaricide, registered for use in ostriches in terms of Act 36 of 1947 may be used.

The ostriches are kept in devegetated camps after acaricide treatment to prevent re-infestation with ticks. MI rodent control programmes must be approved by the National Director Animal Health. The program must have a plan detailing all rodent control sites, the poison to be used and frequency of inspection of such sites. Records must be available on request.

1.4 Residue prevention

No residue forming substances are authorised for use in ostriches. If any residues are discovered in the abattoir monitoring program, the result would be deregistration of the holding.

2. COMMERCIAL POULTRY

It is a standard practise in South Africa that all commercial poultry are vaccinated against Newcastle disease using La Sota and Hitchner B1 vaccine. Ulster strain is also registered.

3. BACKYARD/VILLAGE POULTRY

It is standard practice in South Africa that all backyard poultry within 3km radius of abattoirs are vaccinated using lentogenic vaccines. *(This practice is financed and implemented by the industry in conjunction with the state veterinary service).*

4. VACCINATION IN PIGEONS

Vaccination policy already implemented by pigeon racing industry.

ADDITIONAL CONTROL MEASURES (new proposal)

During outbreaks of Newcastle disease at the holding/farm the following measures are taken:

1. *Within a protection zone with a radius of at least 3 km around tire outbreaks*

- all holders of poultry, ostriches, pigeons etc. are required to have their flock examined twice a week by an authorised Veterinary official.
- the transfer and spreading of poultry litter and manure is prohibited.
- all birds (including pigeons) held in establishments situated within 3km of an outbreak will be confined and vaccinated.

2. Surveillance zone of l0km radius

Within a surveillance zone of at least 10km (containing the protection zone) around the outbreaks, all holders of poultry are required to have all the poultry in their establishments examined once a week by an authorised Veterinarian

3. Slaughter out policy

Slaughter out policy shall be applied in the identified epidemiological unit during an outbreak.

4. Vaccination policy

Compulsory vaccination is still under negotiation.

Conclusion

The control measures described, outlines the South African strategy to achieve the same purpose as expressed in the EC Directive i.e. to minimise risks to enable the guarantees required to facilitate trade. Our request is that the Commission will judge our proposal with this view and maintain realism in judging the level of sanitary protection we are willing to offer and guarantee.

EXPERIMENTAL INFECTION OF VACCINATED SLAUGHTER OSTRICHES WITH VIRULENT NEWCASTLE DISEASE VIRUS

ND V-strain Poultry outbreak: MDT = 47-48h, IVPI = 2,17, ICPI =1,8} @ EID_{50} ; oral, tracheal, nasal and ocular routes

Ostriches:- Minimum weight 90kg; older than 10 months.
"Unvaccinated": No known history of exposure to NDV/vaccines.
"Vaccinated": Live La Sota at six weeks and 10 weeks Inactivated La Sota 30 days before challenge.
Facility: High biosecurity

Samples
(i) Liver, spleen, kidney as an organ pool.
(ii) Muscle (fresh).
(iii) Muscle (after 24h chilling).
(iv) Bone-marrow.
(v) Trachea and Lung (pool)
(vi) Gastro-intestinal tract (pool)
(vii) Choanal & cloacal swabs (clinically affected birds).

All samples were taken from birds that died of clinical NDV; as well as from all survivors when slaughtered 14 days after the last mortality.

NDV IN SLAUGHTER OSTRICHES - BIOSECURE FACILITY:

Results

Unvaccinated			**Vaccinated**		
1. Signs: 10/10 clinically sick, 2/10 died, 8/10 recovered			1. Signs: No clinical signs/deaths		
2 Virology: NDV recovered only from two dead birds			2. Virology: No NDV found in any sample at slaughter		
3. Serology			3. Serology		
25/10/96		19/11/96	25/10/96		19/11/96
11	0,067	1,128	1	0,448	1,385
12	0.071	1,195	2	0,895	1,102
13	0,097	1,153	3	0,275	1,336
14	0,141	1,218	4	0,506	1,294
15	0,876	1,286	5	0,687	1,162
16	0,124	1,247	6	0,109	1,266
17	0,111	1,230	7	1,056	1,352
18	0,097	1,242	8	0,954	1,398
19	0,405	Dead	9	0,176	1,316
20	0,086	Dead	10	0,583	1,356

NDV/OSTRICHES: BIOSECURITY ASPECTS

SA Directorate of Animal Health
SA Poultry Association
OVI Ostrich Unit

Possible Mechanisms of NDV - dissemination	**Biosecurity Measures**
1.Wind-borne:	1 km buffer zone vaccination of all poultry and racing pigeons - La Sota
2. Wild birds:	Pelleted subsistence ration with very low % grain; fed once a day @ 0,5 kg/bird that should be finished within 20-30 min. Water in water troughs disinfected @ viricidal level of 0,5% during experimental period. No water available for 24h after infectious challenge.
3. People and fomites:	Access control: Point duty by OVI **security personnel** **Information/warning signs** **Designated worker*** Abattoir workers informed; strict **washing/disinfection procedures.** **Footbaths** @ access points with virucidal disinfectant. -**Daily faeces** removal by * for incineration with carcasses etc. Drainage ditch as well as whole surface area disinfected with **chlorine lime** upon completion of experiment. Slaughter birds moved with **trailer** to abattoir; disinfected afterwards

NDV IN OSTRICHES: EXPERIMENTAL CHALLENGE OF SLAUGHTER BIRDS IN AN OPEN, FEEDLOT AREA

Summary of observations:

Group 1 = <45 days since vaccination.
Group 2: 59-102 days since vaccination.
Group 3: 136+ days since vaccination.

Week 1

[D:O]: = 13/10/97 (Monday)

Prechallenge bleed, challenge of all three experimental groups with 10^6 EID_{50} using oral, ocular and nasal routes.

[D 4:]: = 17/l0/97 (Friday)
± 20% of birds in Groups 1 and 2 show clinical signs i.e. eyescratching, coughing. [Whole week rainy and overcast].

Week 2

[D:9] = 22/10/97 (Wednesday)
Severe, typical NDV nervous signs in 2 birds from Group 3; also 2 birds dead from this group.
Coughing of ± 3 birds in each of Groups 1 and 2.
Carcasses of affected birds = general congestion, but no typical (poultry) haemorrhages.
Most birds slaughtered on [D:7] (20/10/97) and [D:9] displayed severe splenomegaly with marked white pulp hyperplasia, some also on [D:11].

Week 3

Some birds show Intermittent coughing (several have chronic airsaccullitis lesions on slaughter inspection. The splenomegaly and white pulp hyperplasia noticed last week have reverted back to the normal picture.

Slaughter days [D:14] = 27/10/97; [D:16] = 29/10/97, [D:8] = 31/10/97.

Week 4

Nothing notable;
[D:21] = 31/11/97 Bleed all remaining birds;

[D: 24] = 6/11/97 slaughter 17 birds

Week 5

Nothing notable;
[D: 28] = 10/11/97 Bleed all remaining birds; on
[D: 30] = 12/11/97 slaughter last 17 birds

QUANTITATIVE RISK ASSESSMENT OSTRICHES IN SOUTH AFRICA

SADC - funded (South Africa, Botswana, Namibia, Zimbabwe)
Emphasis on the risk of NDV and CCHF transmission with the export of ostrich MEAT
Rationale to identify risk factors that can be minimised in terms of guarantees required by trade partners
ID of risk factors and variables on *farm-to-fork* approach
Assumption that zero-risk is unattainable
Approach in terms of OIE-guidelines, SPS-agreement and requirements of existing EC Directive for poultry

OSTRICH MEAT: MAJOR FACTORS TO BE ASSESSED

FARMING PRACTICES
CONTROL MEASURES :

- on farm
- procurement of birds
- transport
- at abattoir
- during processing/deboning/packaging

COMPARATIVE RISK

- existing control measures vs. alternatives (e.g. zoning, farm registration)
- sampling methods (on farm, quarantine area, at abattoir)

COMPARATIVE RISK

- existing control measures vs. requirements of EC Directive
- requirements for protected and surveillance zone (3 & 10 km)
- procedures already applied in South Africa to guarantee level of protection needed by trade partners vs. EC requirements for LOP

RISK FACTORS WITHIN PROTECTION ZONE

– risk associated with abattoir workers (own chickens, source of virus transmission)

- backyard chickens
- vaccination coverage
- surveillance/monitoring procedures
- movement control

SOUTH AFRICAN OSTRICH AUDIT SYSTEM

Aim:

The objective of the audit system is a primary guarantee on animal health as well as quality of the product.

Organisations involved:

Producer
Directorate Animal Health
Processor
Directorate Veterinary Public Health

Identification system:

⇒ A tag containing a bar-code number, is applied to the neck skin of the bird, at vaccination.
⇒ The tag is applied under supervision of a vaccination official, who scans the bar-code number with a hand held computerised scanner.
⇒ This number is linked to a data base noting owner, registered holding, name of official, vaccination data.
⇒ At the end of each day, information in the scanner is downloaded into a computer.

Control

A. LOCAL: STATE VET

- Regular on-site visits to export units
- Data base check
- Modem to Central Directorate

B. LOCAL: ABATTOIR

Receive for slaughter only if:
- Data base correct
- Vaccination according to order
- Health status good
- From registered holdings

C. CENTRAL VETERINARY PUBLIC HEALTH

- Ante Mortem inspections
- Data basis correct
- Export certificate

D. CENTRAL : DIRECTORATE ANIMAL HEALTH

- Receives data from local state vet
- Audits for correctness

DATA BASE
EXPORT SLAUGHTER BIRD CERTIFICATE

AUTHORISED PERSON
DATE
REGISTERED HOLDING
OWNER
ADDRESS
BIRD ID
AGE
NO BIRDS IN FLOCK
ORIGIN
HEALTH STATUS
GOOD
FAIR
POOR
CHANGE OF OWNERSHIP

VACCINATION

	DATE	TYPE	VOLUME	BATCH NO
INJECT				
	DATE	TYPE		BATCH NO
EYE DROP				

TREATMENTS

	DATE	TYPE	DATE	TYPE
ANTIBIOTICS				
DEWORMING				
DIP				

BOOSTER DATE

COMMENTS

ORIGINAL CONTRIBUTIONS

ACUTE PANCREATITIS IN CHICKENS DUE TO NON-VIRULENT NEWCASTLE DISEASE VIRUS

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This paper will be published in Veterinary Record during 1998.

QUALITY CONTROL OF SEROLOGICAL TESTS IN FRENCH LABORATORIES

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Introduction

The French diagnostic laboratories are in the process of obtaining accreditation. The COFRAC is the French organisation in charge of the accreditation. At the international level it has signed the EAL (European co-operation for Accreditation of Laboratories) multilateral agreement and the EAC (European Accreditation of Certification) agreement.
The most important laboratory involved in avian diagnosis in France has been accredited for two years.
Beyond this scope, the French diagnostic laboratories are subjected every two years to a quality control of serological tests :

- Newcastle disease, by haemagglutination inhibition (HI)
- Avian influenza, by agar gel precipitation (AGP).

Material and methods

Materials
In 1997 the dispatching of samples was as followed :

For Newcastle disease, 29 sera were sent to each participant. 26 sera were identical for every laboratory and 3 could vary.

Each group of sera was composed of :
- 2 samples of a positive serum (titre 256) and 2 samples of each dilution of this serum (factor 2) from 2 to 256
- 8 different negative sera
- 3 other sera which titres were either 1024, 64 or <4

For Avian Influenza, 28 sera were sent to each laboratory. 26 sera were identical for every laboratory and 2 sera could differ.

Each group of sera was composed of :

- 4 repetitions of a high positive serum,

- 4 repetitions of each dilution of the above serum (1/2, 1/4, 1/8, 1/16),
- 6 repetitions of a negative serum,
- 2 other sera either positive or negative.

Method
The organisation of the assay follows the presentation published before (Proceedings of the 1st & 2d Annual meetings of the National Newcastle Disease and Avian Influenza Laboratories in 1993 & 94).

The serological techniques used, were those published in details by the COFRAC :

- - Detection of Newcastle HI antibodies (IS 280)
- - Detection of Avian Influenza precipiting antibodies (IS 300)

The criteria analysed were :
- Acknowledgement of receipt
- Respect of the deadline to send the results (15 days)
- Precision on the technique (IS 280, IS 300) and reagents used
- The results of each laboratory were analysed by the following parameters :
  Fidelity, repeatability, coherence in dilution, specificity

The results obtained for each parameter were compared to expected results given by the national reference laboratory.
When a laboratory fails for at least one parameter it has to participate in a second test.

Results

Table 1 : Number of laboratories participating

	Newcastle Disease	Avian Influenza
1993	22	15
1995	19	10
1997	14	8

Table 2 : Percentage of laboratories obtaining good results after the 1st test

	Newcastle Disease	Avian Influenza
1993	82	73
1995	68	80
1997	93	100

Table 3. Percentage of laboratories obtaining good results after the 2nd test

	Newcastle disease	Avian Influenza
1993	95	90
1994	100	80
1997	N.F.	100

N.F.= Not finished

Discussion

The number of laboratories which participated in these quality controls decreased each time. This may be due to the fact that the implementation of the quality assurance system is very onerous in time and in cost. Another reason may be the fact that more and more laboratories used ELISA tests for the assessment of the vaccination response of commercial flocks.

Attention has to be drawn to the improvement in the number of diagnostic laboratories giving good values at the first test (Table 2).

COMPARISONS OF HAEMAGGLUTINATION INHIBITION TESTS FOR H5 AND H7 AVIAN INFLUENZA IN DIFFERENT EU NATIONAL LABORATORIES

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Introduction

One of the functions and duties of the Community reference laboratory for Newcastle disease is to organise "periodical comparative tests in diagnostic procedures at Community level". To fulfil this duty a simple test of the reproducibility in the National Laboratories of the haemagglutination inhibition [HI] test for the detection of H5 and H7 influenza virus antibodies was organised.

Materials and methods

Sera

A series of sera, **A-L**, designed to give a range of haemagglutination inhibition titres was prepared at the reference laboratory and freeze-dried. The sera were all from chickens and had the following properties:

Serum A	negative control antiserum used by Reference Laboratory
Serum B	standard positive control antiserum for H5 (to H5N3)
Serum C	against A/tern/S. Africa/61 (H5N3)
Serum D	against A/chicken/Pennsylvania/1/83 (H5N2)
Serum E	serum from an SPF chicken (May 88)
Serum F	against A/chicken/Scotland/59 (H5N1) [***NB*** *N cross reacts with H7N1 antigen*]
Serum G	against A/turkey/Ontario/7732/66 (H5N9)
Serum H	against A/turkey/England/50-92/91 (H5N1) [***NB*** *N cross reacts with H7N1 antigen*]
Serum I	against A/England/247/96 (H7N7)
Serum J	against A/African starling/983/79 (H7N1)
Serum K	against A/chicken/Pakistan/477/95 (H7N3)
Serum L	standard positive control antiserum for H7 (H7N1)

Antigens

Antigens were the following viruses inactivated with ß-propiolactone:

H5 ANTIGEN: A/ostrich/Denmark-Q/72420/96(H5N2)

H7 ANTIGEN: A/African starling/England-Q/79(H7N1)

The H7 antigen was freeze dried, the H5 antigen wet.

Methodology

The sera and antigens were dispatched to the National Laboratory of each EU country with the following instructions:

Accompanying this letter you will find 12 vials, labelled A-L, contain chicken antisera. The others contain Antigen 1(H7) and Antigen 2 (H5) and are labelled accordingly. Vials A, B, and L and Antigen 1 should be reconstituted with 1 ml of distilled water and vials C to K reconstituted with 0.5ml distilled water. Antigen 2 (H5) is in the form of wet stock.

The tests we would like you to carry out are:-

1. *Do haemagglutination inhibition (HI) tests on the antisera A-L using the antigens used in your laboratory and the method you would normally use.*

2. *Do a haemagglutination (HA) tests on the H5 and H7 antigens using your normal technique.*

3. *Following the procedures in Annex III Chapters 5 and 6 of Directive 92/40/EEC prepare 4 HA units of the H5 and H7 antigens and use in HI tests with sera A-L following the 92/40/EEC protocol for HI tests.*

If your normal method is to follow the 92/40/EEC protocol exactly it is still necessary to do two lots of tests using your antigens and the antigens supplied.

Abbreviations
The abbreviations used for countries in this paper are the two letter abbreviations used for Email addresses [http://www.crayne.com/victory/couontry.html]. Except for GB - Great Britain and NIr - Northern Ireland. RL is used for the Community Reference Laboratory

Results and Discussion
Haemagglutination titration
The titration of the antigen is extremely important in this type of exercise as any widely differing titre by any laboratory should affect the HI titres obtained relative to other laboratories. In Directive 92/40/EEC it is recommended that accurate HA measurement is obtained by starting from a close range of initial dilutions. The results suggest this was definitely done by three laboratories BE, FR and NL. For both antigens the consensus titre was 256 [2^8] with 9/16 and 7/16 laboratories returning that titre for H5 and H7 respectively; the geometric mean titres were close to the consensus, 268 for H5 and 232 for H7. All laboratories were within one dilution of the consensus with the exception of IT which gave titres too low [64 for H5 and 32 for H7], and two laboratories with titres too high NIr [1024 for both] and

Table 1. Haemagglutination titration results using H5 antigen

Country	HA titre
RL	128
AT	256
BE	640
DK	512
FI	256
FR	430
GB	128
NIr	1024
GR	256
DE	256
IE	256
IT	64
NL	256
PT	256
ES	256
SE	256
Mean titre: 268	**Consensus titre:** 256

Table 2. Haemagglutination titration results using H7 antigen

Country	HA titre
RL	128
AT	256
BE	448
DK	512
FI	256
FR	445
GB	128
NIr	1024
GR	256
DE	256
IE	256
IT	32
NL	144
PT	128
ES	256
SE	256
Mean Titre: 232	**Consensus Titre:** 256

BE [640 for H5]. These results should lead to underestimation of the antisera titres by the IT laboratory relative to the other laboratories and over estimation by NIr

[both antigens] and BE [for H5]. As can be seen from the analyses of the results this appears to be the case for IT and BE, but the relative results for NIr do not appear to have been substantially affected.

Comparison of HI results using the protocol in Directive 92/40/EC - H5
The results supplied by each laboratory for H5 antigen are shown in Table 3. Overall, all the H5 positive sera, B-D and F-H were recorded as positive by each laboratory and the H5 negative sera A, E, I-L were recorded as having titres <16 by each laboratory, with the exception of a titre of 16 recorded for serum K by FI.

Evaluation of the individual results for each serum with H5 antigen for each laboratory relative to the mean or consensus titres are shown in Tables 4-6. For comparisons with the geometric mean titres 5 laboratories recorded no titres outside the acceptable test limits of one dilution; in total 27/192 titrations (14.1%) were outside the one dilution limit. For comparative purposes the consensus titre (Table 5) is probably a better guide as it is not influenced by exceptionally high or low results produced by a single laboratory and the results relative to the consensus titres are shown in Table 6. In this case 9/16 of the laboratories had no titres outside the one dilution limit and 16/192 (8.3 %) titres in total were outside this limit.

In Table 7 the overall agreement for each laboratory with each of the other laboratories for H5 titrations is summarised by comparing the number of sera with two or more dilutions different. It is difficult to assess what level represents significant discrepancies between two laboratories, but if an arbitrary level of 4 or more sera showing differences of two or more dilutions is taken, it can be seen from the highlighted numbers in Table 7 that differences of this magnitude are associated with four laboratories: FI which reached this level with 9 other laboratories, SE with 10 laboratories, IT with 5 laboratories and BE with 5 laboratories. For BE and IT the differences in antigen titration probably accounted for the variations with other laboratories. The FI comparisons were hampered by the titres, 4-16, obtained with sera I-L which most other laboratories recorded as <2. The results for SE were consistently higher than those reported by most other laboratories and since similar variation was not reported with H7 results (see below) this suggests an antigen preparation problem rather than a titration reading problem may have been the cause.

Table 3. H5 HI results obtained by National Laboratories using the protocol in Directive 92/40/EEC

Serum	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE
A	<2	<2	<2	<2	<2	2	<2	<2	<2	<2	<2	<2	<2	<8	<2	<8
B	64	256	512	128	128	128	128	128	128	128	256	64	128	256	256	512
C	64	256	256	128	128	128	64	128	128	128	128	64	128	128	ND	512
D	64	128	256	128	64	128	128	128	64	128	128	64	128	128	128	256
E	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<8	<2	8
F	256	256	512	256	256	256	256	512	256	256	256	64	256	512	512	1024
G	32	32	128	32	32	32	32	32	64	32	16	32	32	64	64	64
H	128	128	512	256	128	128	128	256	128	128	256	64	256	256	256	512
I	<2	<2	<2	<2	4	<2	<2	<2	<2	<2	<2	<2	<2	<8	<2	<8
J	<2	<2	<2	<2	4	<2	<2	<2	<2	<2	4	<2	<2	<8	<2	<8
K	<2	2	<2	<2	16	<2	2	8	8	<2	4	8	2	<8	2	<8
L	<2	<2	<2	<2	8	<2	<2	<2	<2	<2	<2	<2	<2	<8	<2	<8

Table 4. H5 results obtained by National Laboratories using the protocol in Directive 92/40/EEC titres outside one dilution of mean titre

Serum	RL *	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE	mean
A																	**<2**
B	64		512									64				512	**177**
C																512	**128**
D			256													256	**122**
E																8	**<2**
F			512					512				64		512	512	1024	**154**
G			128								16						**42**
H			512													512	**194**
I					4												**<2**
J					4						4						**<2**
K					16			8	8		4	8					**<2**
L					8												**<2**
Number	**1**	**0**	**5**	**0**	**4**	**0**	**0**	**2**	**1**		**3**	**3**	**0**	**1**	**1**	**6**	

*not included in calculating the mean titre

Table 5. H5 results obtained by National Laboratories using the protocol in Directive 92/40/EEC consensus titres and variations by laboratory.

	Haemagglutination inhibition titres										
Serum	**<2**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**
A	**16**										
B							IT, *RL*	**8**	AT, IE, PT, ES	BE, SE	
C							GB, IT, *RL*	**9**	AT, BE	SE	
D							FI, GB, IT, *RL*	**10**	SE		
E	**15**			SE							
F							IT		**10**	BE, NIr, PT, ES	SE
G					IE	**10**	GR, PT, ES, SE	BE			
H							IT	**7**	**6**	BE, SE	
I	**15**		FI								
J	**14**		FI, IE								
K	**7**	AT, NL, ES	IE	NIr, GR, IT	FI						
L	**15**			FI							

[a]Number of laboratories with this titre.

Table 6. H5 results obtained by National Laboratories using the protocol in Directive 92/40/EEC titres outside one dilution of consensus titre

Serum	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE	consensus
A																	**<2**
B			512									64				512	**128**
C																512	**128**
D																	**128**
E																8	**<2**
F												64				1024	**256**
G											16						**32**
H																	**128/256**
I					4												**<2**
J					4						4						**<2**
K								8	8		4	8					**<2**
L					8												**<2**
Total	**0**	**0**	**1**	**0**	**3**	**0**	**0**	**1**	**1**	**0**	**3**	**3**	**0**	**0**	**0**	**4**	

Table 7. H5 results obtained by National Laboratories using the protocol in Directive 92/40/EEC comparison of variation between laboratories - number sera with titres two or more dilutions different.

	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE
RL	*	2	5	0	4	0	0	1	1	0	4	2	0	1	1	6
AT		*	2	0	4	0	1	1	1	0	1	4	0	0	0	3
BE			*	2	8	3	4	3	4	3	3	7	2	0	0	1
DK				*	4	0	0	1	4	2	2	3	0	0	0	4
FI					*	4	4	3	3	4	3	4	4	1	4	7
FR						*	0	1	1	0	2	2	0	0	0	5
GB							*	1	1	0	1	2	0	0	0	5
NIr								*	0	1	1	1	1	0	1	3
GR									*	1	1	1	1	0	1	5
DE										*	2	2	0	0	0	5
IE											*	3	1	1	2	4
IT												*	3	3	4	6
NL													*	0	0	4
PT														*	0	1
ES															*	1
SE																*

Comparison of HI results using the protocol in Directive 92/40/EC - H7

The results supplied by each laboratory for H7 antigen are shown in Table 8. Overall, all the H7 positive sera, I-L, were recorded as positive by each laboratory and the H7 negative sera A-E and G were recorded as having titres <16 by each laboratory (usually <2), with the exception of titres of 16 recorded for serum G by FR and SE.

Sera F and H were prepared against H5 viruses. but both the viruses used had N1 neuraminidase in common with the H7 antigen supplied. Anti-neuraminidase antibodies may prevent haemagglutination by steric hindrance and give false positive results in HI tests. Titres with H7 antigen for serum F ranged from 16 (DK, GB and IE) to 256 (BE) and for H from 4 (RL and IE) to 128 (BE and ES). All laboratories recorded a positive (16 or greater) H7 titre for serum F and 11/16 laboratories recorded a positive H7 titre for serum H.

Only three of the laboratories (AT, DK and DE) had no results outside one dilution of the mean titre for H7 antigen of each serum, the highest number outside this criterion was 5 sera by GB and BE. Overall 37/191 (19.4 %) results were outside one dilution of the mean titre for each serum. If sera F and H were excluded this fell to 25/159 (15.7%), but did not increase the number of laboratories with no results outside the range (Table 9).

There was a closer overall relationship to the consensus titres (Table 10), although still only the same three laboratories had no results outside one dilution of the consensus titre for any antiserum, although this did improve to 7 if sera F and H were discounted (Table 11). In total 25/191 (13.1 %) titrations were outside one dilution of the consensus titres, but this fell to 16/159 (10.1 %) if results for sera F and H were ignored (Table 11).

The overall agreement for each laboratory with each of the other laboratories for H7 titrations is summarised in Table 12 by comparing the number of sera with two or more dilutions different. If an arbitrary level of 4 or more sera showing differences of two or more dilutions is taken as a significant difference the total number fulfilling this criterion is about the same as the H5 titrations, but in this case they are not so clearly related to a few laboratories. As expected, probably because of the antigen titration, IT showed a high level of difference with 7 other laboratories falling within the criterion, but GB showed this level with 8 laboratories, Belgium 6, Spain 6 and RL 5 without obvious explanation, although some of the differences were boosted by the variations in titres obtained with sera F and H.

Table 8. H7 HI results obtained by National Laboratories using the protocol in Directive 92/40/EEC

Serum	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE
A	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<8	<2	<8
B	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<8	<2	<8
C	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	8	<2	<2	<8	ND	<8
D	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<8	<2	<8
E	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<8	<2	<8
F	32	32	256	16	32	32	16	128	64	32	16	32	32	128	128	32
G	<2	<2	<2	<2	2	16	2	<2	<2	<2	2	<2	<2	<8	2	16
H	4	16	128	16	16	32	8	64	32	16	4	8	8	32	128	16
I	128	512	1024	512	256	512	128	512	256	256	512	128	512	512	1024	512
J	512	1024	2048	1024	512	512	256	1024	256	1024	1024	512	256	1024	2048	2048
K	256	512	1024	512	256	256	128	1024	128	512	512	32	512	1024	1024	512
L	128	512	512	512	128	256	128	512	256	512	512	32	256	512	512	512

Table 9. H7 results obtained by National Laboratories using the protocol in Directive 92/40/EEC titres outside one dilution of mean titre

Serum	RL *	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE	mean
A																	**<2**
B																	**<2**
C											8						**<2**
D																	**<2**
E																	**<2**
F			256					128						128	128		**44**
G						16										16	**2**
H	4		128				8	64			4	8	8		128		**21**
I	128		1024				128					128					**406**
J			2048				256		256				256		2048	2048	**776**
K			1024				128	1024	128			32		1024	1024	512	**388**
L	128				128		128					32					**308**
Number	**3**	**0**	**5**	**0**	**1**	**1**	**5**	**3**	**2**	**0**	**2**	**4**	**2**	**2**	**4**	**3**	

*not included in calculating mean titre

Table 10. H7 results obtained by National Laboratories using the protocol in Directive 92/40/EEC consensus titres and variations by laboratory.

	Haemagglutination inhibition titres											
Serum	**<2**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**
A	**16**[a]											
B	**16**											
C	**16**											
D	**14**			IE								
E	**16**											
F					DK, GB, IE	**8**	GR	PT, ES, NIr	BE			
G	**10**	FI, GB, IE, ES			FR, SE							
H			*RL,*IE	GB, IT, NL	**5**	FR, GR, PT	NIr	BE, ES				
I								*RL,*GB, IT	FI, GR, DE	**8**	BE, ES	
J									GB, GR NL	*RL*, FI, FR, IT	**6**	BE, ES, SE
K						IT		GB, GR	*RL,* FI, FR	**6**	BE, PT, ES	
L						IT		*RL,* FI, GB, NL	FR, GR	**9**		

[a]Number of laboratories with this titre.

Table 11. H7 results obtained by National Laboratories using the protocol in Directive 92/40/EEC titres outside one dilution of consensus titre

Serum	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE	consensus
A																	**<2**
B																	**<2**
C											8						**<2**
D																	**<2**
E																	**<2**
F			256					128						128	128		**32**
G						16										16	**<2**
H	4		128					64			4				128		**16**
I	128						128					128					**512**
J							256		256				256				**1024**
K							128		128			32					**512**
L	128				128		128					32					**512**
Number	**3**	**0**	**2**	**0**	**1**	**1**	**4**	**2**	**2**	**0**	**2**	**3**	**1**	**1**	**2**	**1**	

Table 12. H7 results obtained by National Laboratories using the protocol in Directive 92/40/EEC comparison of variation between laboratories - number sera with titres two or more dilutions different.

	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE
RL	*	3	6	3	1	3	0	5	1	2	3	2	1	5	5	5
AT		*	2	0	1	1	4	2	2	0	2	3	1	1	2	1
BE			*	2	6	4	6	0	5	3	3	6	3	1	0	3
DK				*	1	1	4	2	3	0	2	3	1	1	2	1
FI					*	1	0	4	0	1	3	2	0	3	6	3
FR						*	3	3	0	1	3	5	2	3	5	1
GB							*	6	2	3	5	2	2	6	6	5
NIr								*	2	2	3	5	3	0	0	3
GR									*	2	5	3	2	2	4	3
DE										*	1	2	1	1	3	1
IE											*	4	1	2	2	3
IT												*	3	5	5	5
NL													*	3	3	2
PT														*	1	2
ES															*	3
SE																*

Results obtained by National Laboratories using their own protocols
The laboratories were asked to carry out titrations using their own protocols and antigens. Three laboratories, AT, GR and ES kept no stocks of H5 antigen and were therefore unable to complete that part of the exercise. The results produce by the National Laboratories are recorded in Tables 13 and 14. As with the Directive 92/40 protocol and supplied antigen, generally each laboratory produced negative results for negative sera and positive results with positive sera. The exceptions being positive H5 titres of 32 recorded for serum K by FI, NIr and DE and a titres of 64 and 32 for serum J by FR and IE respectively, H7 titres of 16 for sera Band C by FR and the problems caused by sera F and H in H7 titrations. However. even without more detailed analysis enormous variations in titres can be seen between different laboratories. For example, H5 titres recorded for serum F varied between 32 and 4096 and H7 titres reported for serum L covered a range of 32-2048.

The problems caused by the N1 neuraminidase antibodies in sera F and H tested against H7 virus were exacerbated when the local protocols and antigens were used (Table 14) presumably as a result of the different H7 antigens used possessing N1 or not. This led to large discrepancies between laboratories, for example with serum H NIr, using an H7N1 antigen recorded a titre of 256, while DE and IE, both using H7N7 antigens, recorded titres of <2 and <8 respectively.

Conclusions

Overall there was some consistency among the laboratories in distinguishing positive from negative sera. However, the comparisons of titres was slightly disappointing and not as good as for Newcastle disease virus in the 1995 exercise.

A disappointing number of laboratories used local protocols which did not conform to directive 92/40/EEC. Laboratories should be reminded that adoption of the directive by a member state placed an obligation on the National Laboratory to adopt the protocols annexed to that directive.

The presence of antibodies to neuraminidase may cause problems in obtaining true HI results. The titre levels obtained with sera F and H with the H7 antigen are indicative of this and this could result in different results in different laboratories, especially since the directive does not specify that a particular virus should be used for antigen production. It may be necessary for H5 and H7 HI tests to use two antigens with different neuraminidases for each subtype.

Table 13. H5 HI results obtained by National Laboratories using their own protocols

Serum	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE
A	<2		<2	<2	<2	<4	<2	<2		<2	<8	<2	<2	<8		<8
B	64		1024	512	1024	256	32	256		512	256	32	128	256		1024
C	64		1024	2048	2048	256	32	256		1024	256	32	256	128		1024
D	64		128	256	256	64	64	128		128	128	32	64	128		128
E	<2		<2	<2	<2	<4	<2	<2		<2	<8	<2	<2	<8		<8
F	256		2048	4096	2048	2048	64	512		1024	256	32	256	512		1024
G	32		128	128	128	64	8	64		128	16	32	32	64		64
H	128		512	512	256	256	64	256		256	256	32	256	256		256
I	<2		<2	2	8	<4	<2	<2		<2	<8	<2	<2	<8		<8
J	<2		<2	<2	8	64	<2	<2		<2	32	<2	<2	<8		<8
K	<2		<2	2	32	<4	<2	32		32	8	8	2	<8		8
L	<2		<2	2	8	<4	<2	<2		<2	8	<2	<2	<8		<8

Table 14. H7 HI results obtained by National Laboratories using their own protocols

Serum	RL	AT	BE	DK	FI	FR	GB	NIr	GR	DE	IE	IT	NL	PT	ES	SE
A	<2	<2	<2	<2	<2	<4	<2	<2	<2	<2	<8	<2	<2	<8	<2	<8
B	<2	<2	<2	<2	<2	16	<2	<2	<2	<2	<8	<2	<2	<8	<2	<8
C	<2	<2	<2	<2	<2	16	<2	<2	<2	<2	<8	<2	<2	<8	ND	<8
D	<2	<2	<2	<2	<2	<4	<2	<2	<2	<2	<8	<2	<2	<8	<2	<8
E	<2	<2	<2	<2	<2	<4	<2	<2	<2	<2	<8	<2	<2	<8	<2	<8
F	32	128	256	128	32	8	8	128	64	<2	<8	32	16	<8	128	32
G	<2	<2	<2	4	4	16	<2	<2	<2	<2	<8	<2	<2	8	2	8
H	4	32	128	64	8	<4	4	256	32	<2	<8	8	8	<8	64	8
I	128	512	1024	2048	256	1024	64	512	256	256	512	32	128	256	1024	512
J	512	1024	2048	2048	512	2048	128	1024	256	512	1024	256	256	512	2048	2048
K	256	512	1024	2048	256	1024	64	1024	128	256	512	32	256	512	1024	1024
L	128	512	512	2048	64	2048	64	512	128	1024	1024	32	256	1024	512	1024

Recommendations

In view of the results obtained in this comparative study the following are recommended:

- All National Laboratories should adopt the protocols described in Directive 92/40/EEC
- All National Laboratories should be fully equipped to identify H5 and H7 influenza viruses and to carry out HI tests to assess antibodies to these two influenza subtypes.
- All National Laboratories should use antigens derived from the same viruses in HI tests. [The viruses to be used for this purpose should be agreed at the next annual meeting of the laboratories and the Reference laboratory undertake to supply two viruses of each subtype to each National Laboratory, either as live virus seeds or inactivated antigen, as demanded by the National Laboratories].

DISCUSSIONS, CONCLUSIONS, RECOMMENDATIONS

DISCUSSION OF THE FOLLOWING TOPICS

- *current definitions of ND and AI*

The differences between the current EU definition and the proposed OIE definition [see Commission contribution above] were discussed. The participants agreed:

i. They could see no reason for increasing the qualifying ICPI from 0.7 to 1.2
ii. A definition of "poultry" should be appended to the definition of ND
iii. Nucleotide sequencing to determine the amino acid sequence at the F0 cleavage site could be added as an alternative for *confirming* disease, but an ICPI test should still be necessary if sequencing results do not confirm the virus as virulent.

- *capacities for diagnostic testing in National Laboratories*

The participants were asked if they considered their laboratory would be capable of dealing with a series of outbreaks of Newcastle disease or avian influenza.

The general consensus of those that commented was that they either considered that they had dealt with a series of outbreaks in the recent past or there was sufficient capacity and/or contingency plans to deal with a large number of outbreaks.

The question of what should be done if the national laboratory was unable to cope, for example in the case of multiple outbreaks of both ND and avian influenza was asked. The representative for the Commission said that the Community Reference Laboratory and the National Laboratories of other Member States should be asked for help.

- *problems associated with current diagnosis*

Various problems associated with current diagnostic procedure were discussed these covered:
– The large number of swab samples [60] that have to be taken and processed for virus isolation from seropositive flocks in a non-vaccinating country.
– The delays caused by virus isolation attempts and virus characterisation often result in welfare problems for broilers near to market weight

For both these points the delays and difficulties appeared to relate to the virus isolation procedures in the Directives which require 7 days incubation in eggs after inoculation and two passages before the sample is considered negative. This could take up to three weeks which is a long time to maintain restrictions on flocks and half the life time of a broiler chicken. Discussion took place on how to reduce the time period, especially to reach a negative diagnosis. The need for two passages in eggs was discussed. It was agreed that while the large majority of viruses isolated are detectable after one passage, for influenza viruses and all viruses isolated from species other than poultry, including pigeons, viruses sometimes need two passages in eggs. It was considered possible that overlapping passages [i.e. doing the second passage after only two days] might be as effective and save some time, but it was difficult to see how this could be evaluated. The use of PCR techniques directly on tissues was seen as a possible solution, but at present, those who had used the techniques considered egg inoculation to be more sensitive. The representative for the Commission considered it may be time to review the procedures specified in the Directives.

- *problems associated with the HI and AGP tests with ostrich sera*

Most laboratories reported experiencing problems with ostrich sera in HI tests due to the agglutination of chicken red blood cells by the ostrich sera. This appears to be a common problem when serum samples are from species of birds distant from chickens. Most laboratories overcome this problem by routine pre-adsorption of the ostrich serum with chicken red blood cells [*add about 25 µl packed chicken red blood cells to 0.5 ml ostrich serum, mix gently and leave to adsorb for one hour, centrifuge red blood cells to a pellet, decant serum and inactivate at 56 ^{o}C for 30 minutes before titrating*]. Alternatively, as required in France, the haemagglutination titre for each serum can be assessed and results interpreted in relation to the HI titre, but, of course, for sera with high HA titres this may mean the HI test is not readable. The other option was to use ostrich red blood cells, but these were rarely available to National Laboratories.

Problems also occur with AGP tests for influenza type A NP antigen with some species of bird. The French National Laboratory had used commercially available ELISA kits as a possible replacement for the AGP test, but found some variations in the tests and concluded that further evaluation was necessary if such ELISA kits were to replace the AGP test.

- *routine use of PCR for influenza viruses of different subtypes*

The requirements for sub-typing avian influenza viruses and the large number of antisera needed were discussed. The suggestion that PCR could be an alternative was considered, but while this was thought to be a possibility, problems such as primers for each subtype etc would need to be overcome.

- *use of reference sera and other reagents in National Laboratories*

The use of reference reagents supplied by the Community Laboratory was discussed. Generally it was felt that certainly for H5 and H7 influenza all laboratories should use the same viruses. The view was expressed that the Community Laboratory should supply sera that could be used to validate Nationally produced antisera rather than supply sera to be used in tests. It was pointed out that for NDV and avian influenza antisera had been supplied for comparative tests which could be used for that purpose.

- *role of the Community Reference Laboratories in 1998*

The role of the Community Reference Laboratory in receiving viruses, organising comparative tests and supplying antisera and antigens to the National Laboratories was discussed. The proposed functions and duties during 1998 were presented to the meeting and these were agreed as what was required by those present.

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