COMMISSION
OF THE
EUSROPEAN COMMUNITY

Directorate General for
Health and Consumer Protection
Sanco E2

SANCO/899/2002 EN

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

HELD IN UPPSALA, SWEDEN
26th-28th APRIL 2001

Edited by Dennis J. Alexander

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PROGRAMME

Place: National Veterinary Institute, Ulls väg 2 B, Ultuna,Uppsala

Thursday, 26 April 2001

09.30-09.50	Opening of the meeting
	Morning session - Chairman: Dr. A. Engvall
09.50-10.30	Report from the EU Reference Laboratory - Dr. D. Alexander
10.30-10.50	Report from the European Commission - Drs. J.M. Westergaard and M. Pittman
10.50-11.05	*Coffee break*
11.05-12.00	Country reports on ND and AI based on responses to Questionnaire - Dr. D. Alexander
12.00-12.20	Discussions related to morning sessions
12.20-13.20	*Lunch*
	Afternoon session - Chairman: Dr. D. Alexander
13.20-14.20	Interlaboratory comparative tests for ND and AI in 2000
14.20-16.00	Original contributions:
	(a) Clinical, gross and microscopic findings in different avian species infected naturally during the H7N1 HPAI and ND epidemics in Italy during 1999 and 2000 - Dr. Franco Mutinelli
15.05-15.30	*Coffee break*
	(b) ND-virus by Swedish scientist
16.00-16.30	Discussion: the definition of Newcastle Disease and matters arising
16.30-18.00	Prevention of infectious poultry diseases (viral diseases and Salmonella)

EU REPORTS

REPORT OF THE EUROPEAN UNION REFERENCE LABORATORIES FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE 2000

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Introduction

Each year the Commission and the Community Reference Laboratory [CRL] agree a specific work plan, which is usually presented to the Annual Meetings of the National Laboratories. At the end of each year the CRL produces a technical report of work done against the work plan for avian influenza [AI] and Newcastle disease [ND]. The present paper is a combination of those technical reports.

I. LEGAL FUNCTIONS AND DUTIES

The functions and duties for AI are specified in Annex V of Council Directive 92/40/EC (Official Journal of the European Communities No L 167 of 22.6.1992). The functions and duties for ND are specified in Annex V of Council Directive 92/66/EEC (Official Journal of the European Communities No L 260 of 5.9.1992).

II. OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2000

(1) Collecting and editing of material for a report covering the annual meeting of National Avian Influenza Laboratories held in Brussels, November 1999.

Work Plan: *Receive and collate submissions during January to March edit and produce report of proceedings by end of May.*

WORK DONE: The usual delay in producing the report due to delay in receiving contributions from participants occurred. The final version of the proceedings was produced in August as Document SANCO/2472/2000 and distributed to participants and other interested parties.

(2) Characterising viruses submitted to the Laboratory by Member States and third countries listed in Commission Decision 95/233/EC (Official Journal of the European Communities N° L 156, p. 76) as amended by Decision 96/619/EC (OJ N° L 276, p. 18). This will include:

For AI:

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

For ND:

a) Determining the intracerebral pathogenicity index (ICPI)
b) Determining basic amino acids at the F0 cleavage site of the virus
c) Antigenic grouping of viruses
d) Limited phylogenetic analyses.

Work Plan:
The number of viruses received will be dependent on the outbreaks occurring and those viruses submitted. The haemagglutinin and neuraminidase subtypes of all influenza viruses submitted will be determined. IVPI tests will be done at the request of the submitting laboratory or the Commission. The amino acids at the haemagglutinin cleavage site of all viruses of H5 and H7 subtype will be deduced by nucleotide sequencing. For selected viruses sequencing will be extended into other areas of the H gene to allow phylogenetic analyses. The identification of all viruses received will be confirmed. All ND viruses will be subjected to antigenic grouping using monoclonal antibodies. ICPI tests will be done if not already assessed in the National Laboratories. Nucleotide sequencing and phylogenetic studies will be carried out on representative viruses of each submission.

WORK DONE: The number of viruses received from all sources in 2000, 704, was the highest number submitted to the CRL [Table 1].

Table 1: Number of submissions to the reference laboratory by year since 1987.

1987	***1988***	***1989***	***1990***	***1991***	***1992***	***1993***
133	*401*	*188*	*113*	*154*	*199*	*294*
1994	***1995***	***1996***	***1997***	***1998***	***1999***	***2000***
385	*605*	*284*	*266*	*305*	*357*	*704*

Of the 704 submissions 413 were AI viruses and 254 paramyxoviruses, of which 249 were APMV-1. Characterisation of the viruses was undertaken and the results are presented in Table 2. Viruses were received from the following EU member countries: United Kingdom, Germany, Italy, Netherlands, Spain, Ireland, Sweden, Denmark, Austria and France and the following non-EU countries: Croatia, Peru, Iran, China, Taiwan, Albania, Syria, Singapore, UAE, Saudi Arabia, Jordan, Norway, Pakistan, and Bulgaria.

The large number of H7N1 viruses received reflected the 413 outbreaks of HPAI in Italy during 2000. In addition to conventional typing of the viruses submitted a total of 365 H7 viruses was subjected to nucleotide sequencing and the amino acids at the haemagglutinin cleavage site deduced. Of these 350 had multiple basic amino acids and therefore were HPAI viruses, 15 had amino acid motifs consistent with virus of low pathogenicity. Extended sequencing was done on 56 representatives of the Italian LPAI and HPAI H7N1 viruses for phylogenetic studies. Three IVPI tests were done at the request of the submitting country.

Table 2: Identification of viruses submitted to the reference laboratory in 2000

Virus identification	Number
Influenza A viruses	***413***
H1N2	2
H3N8	5
H7N1	389
H7N3	1
H9N2	14
H11N9	2
Paramyxoviruses	***254***
APMV-1 [NDV]	249
APMV-2	2
APMV-3	1
APMV-7	2
others	***37***
reovirus	2
herpesvirus	4
IBV	1
untyped	19
virus not viable	11

In addition to identification [and when requested by the submitting country], 39 intracerebral pathogenicity index tests were done on the submitted ND viruses to assess their virulence. All APMV-1 viruses were also assessed using a panel of monoclonal antibodies to determine antigenic and epizootiological relationships [Table 3]. For a number the nucleotide sequence of an area of the fusion protein gene from the signal sequence through the cleavage site was obtained for *in vitro* assessment of virulence and use in phylogenetic studies.

Table 3. Characterisation of avian paramyxoviruses received from different countries.

Country	Number	Characterisation
EU COUNTRIES		
Italy	115	APMV-1[mAb groups 106 x C1, 4 x P, 1 x E, 1 x G, 3 x ?]
UK	29	25 x PPMV-1 2 x APMV-2, 1 x APMV-3, 2 x APMV-7
Germany	1	APMV-7 [reptile]
Northern Ireland	8	APMV-1 [mAb groups 4 x E, 1 x P, 3 x P?]
Ireland	3	APMV-1 [mAb groups 2 x P, 1 x H]
OTHER COUNTRIES		
Norway	1	APMV-1 [G?]
Saudi Arabia	12	APMV-1 [12 x C1]
UAE	32	APMV-1 [12 x C1, 4 x P, 16 x ?]
Jordan	1	APMV-1 [?]
Bulgaria	10	All APMV-1 [10 x C1]
Croatia	3	APMV-1 [1 x B, 2 x E]
Singapore	2	APMV-1 [2 x C1]
Pakistan	3	APMV-1 [3 x E]

(3) Maintain virus repository and distribute viruses from it and reagents necessary for virus characterisation.

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

WORK DONE: The viruses received were added to the repository. Reagent stocks were maintained, at least at previous levels [Table 4] although the demand for reagents was much higher than usual and during the year the following were supplied:

INFLUENZA
SERA : H1 - 7ml, H2 – 7ml, H3 – 7.5ml, H4 – 9ml, H5 - 16.5ml, H6 – 12.5ml, H7 – 16.5ml, H8 – 5ml, H9 – 22ml, H10 – 7ml, H11 – 6.5ml, H12 – 4.5ml, H13 – 4.5ml, H14 – 3.5ml, H15 – 3.5ml each AGID+ve - 14ml.
ANTIGENS: AGID [Beard] antigen - 30ml, H3 - 1ml, H5 - 22ml, H7 315ml, H8 – 2ml, H9 - 111ml, H14 – 2ml, H15 – 2ml.
VIRUSES: Number 0.5ml ampoules H4 x 4, H5 x 4, H7 x 3, H9 x 2, H10 x 2, H14 x 1, H15 x 1.

PARAMYXOVIRUSES
MABS: mAb85 - 11ml; mAb7D4 - 11ml; mAb 161/617 7ml
SERA: APMV-1 antiserum - 76ml; APMV-2 13ml, APMV-3 13ml, APMV-4 1ml, APMV-5 3ml; SPF antiserum 8ml.
ANTIGENS: APMV-1 antigen 80ml, APMV-2 34ml, APMV-3, 28ml, APMV-4 1ml, APMV-6 1ml.
VIRUSES: APMV-1 x 8, APMV-3 x 3 ampoules.

Table 4. Stocks of polyclonal chicken sera and virus antigens for HI tests held at the Reference Laboratory.

Type	Serum		Antigen	
	Quantity[a]	HI titre[b]	Quantity[a]	HA titre[b]
SPF	100	<2		
H5	100	8	25	7
H7	125	8	35	7
SPF	100	<2		
APMV-1	100	8	100[c]	7
APMV-3	100[d]	8	50[d]	8

[a]Number of freeze-dried ampoules containing 1 ml of serum or antigen at the indicated titre. [b]HI and HA titres are expressed as log_2. [c]Ulster 2C. [d]turkey/Engand/1087/82. The SPF serum had an HI titre of <2 to each antigen.

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

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

WORK DONE: Antigens were prepared and dispatched to EU National Laboratories for comparative tests for antigen identification. In addition to the EU National laboratories these antigens were also supplied to laboratories in putative member countries.

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

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

WORK DONE: Since there was no annual meeting in 2000 the reagents were sent out towards the end of 2000. The results were presented at the meeting and are published in these proceedings.

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

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

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

(7) Support by means of information and technical advice National Avian Influenza Laboratories and the European Commission during epidemics.

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

WORK DONE: Staff of the CRL worked in close collaboration with the Italian National Reference Laboratory during the HPAI outbreaks in 2000 and were consulted on numerous occasions by, other National Laboratories, representatives of other Member States and of the Commission.

(8) Prepare programme and working documents for the Annual Meeting of National Avian Influenza Laboratories to be held in 2000.

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

WORK DONE: Preparative planning for the 2001 meeting was done in collaboration with the Commission and representatives of the host country.

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

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

WORK DONE:
The following publications appeared in 2000 relating to the work of CRL.

AVIAN INFLUENZA.

1. ALEXANDER, D.J. & BROWN, I.H. (2000). Recent zoonoses caused by influenza A viruses. OIE Scientific and Technical Review 19, 197-225.
2. ALEXANDER, D.J. (2000) A review of avian influenza in different bird species. Proceedings of the ESVV Symposium on Animal Influenza Viruses, Gent 1999. Veterinary Microbiology 74, 3-13.
3. BANKS, J., SPEIDEL, E.C., McCAULEY, J.W. & ALEXANDER, D.J. (2000). Phylogenetic analysis of H7 haemagglutinin subtype influenza A viruses. Archives of Virology 145, 1047-1058.
4. BANKS, J., SPEIDEL, E.C., HARRIS P.A. & ALEXANDER, D.J. (2000). Phylogenetic analysis of influenza A viruses of H9 haemagglutinin subtype. Avian Pathology 29, 353-360.
5. ALEXANDER D.J., MANVELL R.J. & FROST, KM (2000). Report of the European Union Reference Laboratories for avian influenza and Newcastle disease 1998. Proceedings of the Joint Sixth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Brussels 1999, pp 72-77
6. ALEXANDER D.J. & MANVELL R.J. (2000). Comparative tests for antigen identification in different EU National Laboratories 1999. Proceedings of the Joint Sixth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Brussels 1999, pp 47-52
7. ALEXANDER, D.J. (2000). Epidemiologia influenzy ptaków. In: Materialy Konferencja Naukowa Influenza Ptaków, Pulawy, Poland 1-7.
8. ALEXANDER, D.J. (2000). Diagnostyka influenzy ptaków. In: Materialy Konferencja Naukowa Influenza Ptaków, Pulawy, Poland 22-29.
9. ALEXANDER, D.J. (2000). Zwalczanie influenzy ptaków w unii Europejskiej-Obercny stan prawny i perspectywy. In: Materialy Konferencja Naukowa Influenza Ptaków, Pulawy, Poland 30-35.
10. ALEXANDER, D.J. (2000). The history of avian influenza in poultry. World Poultry Avian Influenza Special November 7-8.
11. ALEXANDER, D.J. (2000). How dangerous are avian influenza viruses for humans? World Poultry Avian Influenza Special November, 11-12.
12. ALEXANDER, D.J. (2000). 15. The role of the International Reference Laboratory for avian influenza. World Poultry Avian Influenza Special November 15-16.
13. CAPUA, I., MUTINELLI, F., MARANGON, S. & ALEXANDER, D.J. (2000). H7N1 Avian influenza in Italy (1999-2000) in intensively reared chickens and turkeys. Avian Pathology 29, 537-543.
14. CAMERON, K.R., GREGORY, V., BROWN, I.H., ALEXANDER, D.J., HAY, A. & LIN, Y.P. (2000). H9N2 subtype influenza A viruses in poultry in Pakistan are closely related to the H9N2 viruses responsible for human infection in Hong Kong. Virology 278, 36-41.

AVIAN PARAMYXOVIRUSES

15. ALEXANDER, D.J (2000). Newcastle disease in ostriches (*Struthio camelus)* – A review. Avian Pathology 29, 95-100.
16. JØRGENSEN, P.H. JENSEN HANDBERG, K., AHRENS, P., MANVELL, R.J., FROST, K.M. & ALEXANDER, D.J. (2000). Similarity of avian paramyxovirus serotype 1 isolates of low virulence for chickens obtained from contaminated poultry vaccines and from poultry flocks. Veterinary Record 146, 665-668.
17. ALEXANDER, D.J. (2000) Newcastle disease and other avian paramyxoviruses. OIE Scientific and Technical Review 19, 443-462
18. ALEXANDER D.J. & MANVELL R.J. (2000). Newcastle disease: situation in Great Britain 1999. Proceedings of the Joint Sixth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Brussels 1999, pp 41-42
19. ALEXANDER D.J., MANVELL R.J. & FROST, KM (2000). Report of the European Union Reference Laboratories for avian influenza and Newcastle disease 1998. Proceedings of the Joint Sixth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Brussels 1999, pp 72-77
20. ALEXANDER D.J. & MANVELL R.J. (2000). Comparative tests for antigen identification in different EU National Laboratories 1999. Proceedings of the Joint Sixth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Brussels 1999, pp 47-52
21. ALEXANDER, D.J., MANVELL, R.J. & GOUGH, R.E. (2000) Newcastle disease – World situation. Proceedings XI Congreso Nacional de Medicina Veterinaria Chile 2000 pp5 [CD].
22. GUTIERREZ-RUIZ, E.J., RAMIREZ-CRUZ, G.T., CAMARA GAMBOA, E.I., ALEXANDER, D.J. & GOUGH, R.E. (2000). A serological survey for avian infectious bronchitis virus and Newcastle disease virus antibodies in backyard (free range) village chickens in Mexico. Tropical Animal Health Production. 32, 381-390.

COMMUNITY STANDARDS CONCERNING QUALITY STANDARDS OF LABORATORIES IN THE AREA OF ANIMAL HEALTH

Working Document SANCO/2437/2001

Jorgen M. Westergaard and Maria Pittman
European Commission, Directorate General for Health and Consumer Protection

Introduction

The measures introduced in the area of animal health in relation to the establishment of the Single market had the objective to ensure a high level of protection of human and animal health within the Community and to further develop animal disease control and eradication measures. With the objective to reach these targets a comprehensive set of EU legislation has been adopted. One part of this legislation covers in particular the control and eradication of O.I.E. list A diseases (the control directives); one part relates to movement of animals and animal products (the trade directives) and one part covers health aspects of aquaculture animals.

A brief review of the legislation will outline the provisions applicable to veterinary diagnostic laboratories specifically referred to in relation to the measures adopted for the control and eradication of O.I.E. list A diseases and furthermore to the provisions relevant to those laboratories which primarily carry out tests of importance for movements of terrestrial and aquaculture animals.

CONTROL AND ERADICATION OF O.I.E. LIST A DISEASES

The current EU legislation governing the control and eradication of O.I.E. list A diseases was by and large adopted during the period 1980-1992 and the legislation is contained in seven directives, see table 1.

Table 1. Information on diseases subjected to control and eradication measures within the context of EU directives

Council Directive	Diseases subject to control and eradication measures
80/217/EEC	Classical swine fever
85/511/EEC	Foot-and-mouth disease
91/67/EEC 93/53/EEC	Infectious Salmon anaemia, Viral haemorrhagic septicaemia, Infectious haematopoietic necrosis.
92/35/EEC	African horse sickness
92/40/EEC	Avian Influenza
92/66/EEC	Newcastle Disease
92/119/EEC	Epizootic haemorrhagic disease of deer, Lumpy skin disease, Rift Valley Fever, Rinderpest, Sheep and goat pox, Swine vesicular Disease, Vesicular Stomatitis
95/70/EC	Haplosporidiosis, Perkinosis, Mikrokytosis, Iridovirosis, Marteiliosis
2000/75/EEC	Bluetongue

The Council Directives listed in table 1 contain requirements concerning National Reference Laboratories of the Member States and the Community Reference Laboratories. In this paper the measures applicable to National Reference Laboratories and the Community Reference Laboratory for Avian Influenza will be used as a prototype. In Council Directive 92/40/EEC introducing measures for the control of avian influenza the requirements for a national reference laboratory are those shown below:
"The national avian influenza laboratory in each Member State shall be responsible for co-ordinating the standards and diagnostic methods laid down in each avian influenza diagnostic laboratory within the Member State. To this end:

a) they may provide diagnostic reagents to national laboratories;

b) they shall control the quality of all diagnostic reagents used in that Member State;

c) they shall arrange comparative tests periodically;

d) they shall hold isolates of avian influenza virus from cases confirmed in that Member State.

e) they shall ensure that the confirmation of positive results obtained in regional diagnostic laboratories."

The same directive lists in some details the functions and duties of the Community Reference Laboratory for avian influenza. The functions and duties are given in Annex I.
An important function and duty of the National and the Community Reference Laboratory is to "arrange comparative tests periodically". In this context it must be emphasised that inter-laboratory comparative tests are being carried out for a variety of reasons such as:

a) to determine the capability of a laboratory to conduct specific diagnostic tests;

b) to check or certify the performance of individual operators;

c) to check or certify the calibration of instrumentation;

d) to harmonise existing test methods;

e) to evaluate new test methods;

f) to assign values and ranges to standard materials;

g) to resolve inter-laboratory differences.

At present (year 2001) the EU has adopted provisions for the operation of nine Community Reference Laboratories, see table 2, and all nine laboratories perform annually inter-laboratory test comparisons. The results of these tests are discussed at meetings attended by participants of the National Reference Laboratories and published in Reports issued by the Community Reference Laboratory responsible for the test. The Community financial annual contributions made available for the operation of a CRL vary from 40.000 - 185.000 EURO.

Table 2. List of Community Reference Laboratories in operation and their geographical location

Community Reference Laboratory for	Location
African Horse Sickness	Algete, Spain
Avian Influenza	Weybridge, United Kingdom
Bluetongue	Pirbright, United Kingdom
Bivalve molluscs Diseases	Tremblade, France
Classical Swine Fever	Hannover, Germany
Fish Diseases	Aarhus, Denmark
Newcastle Disease	Weybridge, United Kingdom
Rabies serology	Nancy, France
Swine Vesicular Disease	Pirbright, United Kingdom

From the above it can be noted that the legislation stipulates the functions and duties of National and Community Reference Laboratories for a number of O.I.E. List A diseases and certain diseases of aquatic animals, but there is no specific reference or requirements to laboratory quality evaluation (accreditation).
With regard to foot-and-mouth disease laboratories the legislation, Council Directive 85/511/EEC clearly indicates the bio-security requirements for laboratories handling live FMD virus and for laboratories producing FMD vaccine.
For reference laboratories, other than FMD laboratories, the legislation does not stipulate specific requirements to bio-security and equipment. In an attempt to assist Member States on this matter, however, minimum requirements for the equipment and personnel of National Swine Fever Laboratories have been drawn up, see Annex II.

DIAGNOSTIC LABORATORIES AND TRADE IN LIVE ANIMALS AND ANIMAL PRODUCTS
With the objective to facilitate trade in live animals and animal products without causing spread of infectious diseases the EU has adopted a number of directives covering animal health requirements for intra-Community trade and importation from third countries. The main directives are listed in table 3.

Table 3. Legislation governing intra-Community trade and importation from third countries in relation to different animal species

Trade Directives	Intra-Community Trade and Importation
64/432/EEC 72/462/EEC	Cattle and pigs
91/68/EEC 72/462/EEC	Sheep and goats
90/426/EEC	Horses
90/539/EEC	Poultry
91/67/EEC	Aquaculture animals

All animals entering intra-Community trade must fulfil well defined animal health conditions and be accompanied by a health certificate. The health certificate is a cornerstone of the legislation as it contains vital health information that often is based on the results provided by diagnostic laboratories. Information on the need for laboratory testing with regard to intra-Community trade is given in table 4.

Table 4. Laboratory testing related to intra-Community trade in cattle, swine, sheep, goats and poultry

Trade Directive	Traded Species	Laboratory Testing Requirements
64/432/EEC	Cattle	Brucellosis, Enzootic Bovine Leukosis, Infectious Bovine Rhinotracheitis
64/432/EEC	Swine	Aujeszky's disease
91/68/EEC	Sheep, Goats	Brucellosis
90/539/EEC	Poultry	Salmonella, Mycoplasma

The diagnostic laboratories carrying out testing for the infectious diseases listed in table 4 are not subject to an inter-laboratory testing programme similar to the one organised by Community Reference Laboratories listed in table 2. Occasionally, however, such tests have been carried out.

QUALITY ASSURANCE (ACCREDITATION) OF DIAGNOSTIC LABORATORIES IN THE ANIMAL HEALTH AREA

The EU legislation has as described above stipulated functions and duties of National and Community Reference Laboratories for a number of infectious animal diseases. The inter-laboratory comparison testing programme has been and is an important tool in ensuring reliable laboratory results. The O.I.E.[1] has prepared guidelines for laboratory evaluation based on the relevant requirements of the International Organisation for Standardisation (ISO) 9000 series of standards and ISO/International Electrotechnical Commission (IEC) Guide 25 as accreditation to these standards should provide sufficient reassurance of the competence of a testing laboratory. More recently a number of the animal health reference diagnostic laboratories have participated, on a voluntary basis, in quality assurance/accreditation schemes. In response to a questionnaire prepared on this topic National Reference Laboratories designated in respect of Avian Influenza, Swine Vesicular Disease and Fish Diseases, have provided information as shown in table 5.

Table 5. The accreditation status of some National Reference Laboratories

Quality Assurance Status (Accreditation)	National Reference Laboratories		
	Avian Influenza	**Swine Vesicular Disease**	**Fish Diseases**
Intend to participate in a scheme	4	4	7
Participate and seeking accreditation	3	5	3
Accredited at present	4	1	5
Not considering accreditation or no information	3	3	0
Total number of National Reference Laboratories	14	13	15

[1] Office International des Epizooties (OIE) (1998) – Guidelines of the Office International des Epizooties for laboratory quality evaluation, for international reference standards for antibody assays and for laboratory proficiency testing. Rev. sci. tech. Off. int. Epiz. 17(2)600-609.

ANNEX I

COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA

The functions and duties of the Community reference laboratory for avian influenza shall be:

1. To co-ordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing avian influenza, specifically by:

   a) typing, storing and supplying strains of avian influenza virus for serological tests and the preparation of anti-sera;

   b) supplying standard sera and other reference reagents to the national reference laboratories in order to standardise the tests and reagents used in the Member States;

   c) building up and retaining a collection of avian influenza virus strains and isolates;

   d) organising periodic comparative tests of diagnostic procedures at Community level;

   e) collecting and collating data and information on the methods of diagnosis used and the results of tests carried out in the Community;

   f) characterising isolates of avian influenza viruses by the most up-to-date methods available to allow greater understanding of the epizootiology of avian influenza and to gain an insight into the epizootiology of the virus and the emergency of highly pathogenic and potentially pathogenic strains;

   g) keeping abreast of developments in avian influenza surveillance, epizootiology and prevention throughout the world;

   h) retaining expertise on avian influenza virus and other pertinent viruses to enable rapid differential diagnosis;

   i) acquiring a thorough knowledge of the preparations and use of the products of veterinary immunology used to eradicate and control avian influenza.

2. To actively assist in the diagnosis of avian influenza outbreaks in Member States by receiving virus isolates for confirmatory diagnosis, characterisation and epizootiological studies. In particular, the laboratory should be able to carry out nucleotide sequencing analysis to allow determination of the deduced amino acid sequence at the cleavage site of the haemagglutin molecule of avian influenza viruses of H5 or H7 subtype.

3. To facilitate the training or retraining of experts in laboratory diagnosis with a view to the harmonisation of techniques throughout the Community.

ANNEX II

MINIMUM REQUIREMENTS FOR THE EQUIPMENT AND PERSONNEL OF NATIONAL SWINE FEVER LABORATORIES

At the Thirteenth Annual Meeting of the National Swine Fever Laboratories (NSFL) held in Alghero, Sardinia in 1996 it was recommended that guidelines should be prepared which state the minimum equipment and personnel required for NSFLs to carry out the diagnostic procedures for classical swine fever (CSF) and African swine fever (ASF).

The diagnostic procedures for CSF are laid down in Annex I and Annex IV of Directive 80/217/EEC whereas the minimum requirements for the equipment and personnel of NSFLs are not specified.

The principal basis for the laboratory diagnosis of CSF is the demonstration of CSF virus, CSF antigen, CSF nucleic acid (not mentioned yet in the directive) and antibodies to CSF virus.

Laboratory containment

CSF virus is regarded as relatively fragile, and does not spread readily by the airborne route. Thus a combination of good microbiological practice in the laboratory and the use of Class I/III microbiological safety cabinets when handling infectious materials should be sufficient to avoid risk of escape. A high containment level laboratory, such as required for work with foot-and-mouth disease virus, is not obligatory. In contrast, where experimental infection of pigs is to be undertaken, these represent a real risk of spread of the virus and they should be housed under conditions of high containment. The CSF diagnosis should be conducted in laboratory rooms which are strictly separated from other laboratories, specially dealing with cattle, sheep or other pig diseases to avoid contamination with other viruses (e.g. BVD virus). The laboratory must be separated from the public access and has to include decontamination facilities (e.g. autoclave), hand-washing facilities and a shower.
Within the CSF laboratory the following separated working areas should exist:

- a clothes change room with shower
- necropsy room with sink for hand-washing
- room for keeping microscopes
- room for keeping deep freezers and liquid nitrogen containers
- working area for cell cultures (if possible separate room)
- working area for infectious material (virus cultivation, detection and isolation)
- working area for serological tests

Laboratory equipment

Isolation of CSF virus from organ materials and blood as well as detection of neutralising antibodies against CSF virus requires to work with cell cultures.
The minimum equipment requirements for the above-mentioned work are:

- two -80°C deep freezers
- two -20 or -40°C deep freezers
- two refrigerators (+ 4°C)
- liquid nitrogen container for cells
- two incubators, one of which is a CO_2 incubator
- two laminar flow cabinets Biosecurity containment (BSC) II
- two inverted microscopes
- one microscope for fluorescent techniques
- two refrigerated centrifuges
- one water purification unit producing water of double distilled water (DDW) quality
- one autoclave
- one sterilisation oven
- instruments for post mortems
- one homogenizer/blender
- one pH meter
- one lyophilization apparatus
- two multichannel pipetes, 6 eppendorf pipetes (10-200µl)
- one water bath (37°/56°C)
- one vortex
- one lab shaker

For the direct demonstration of viral antigen in organ tissues one cryotom for cryostat sections is required.
For the demonstration of antibodies or viral antigen with the ELISA one ELISA system (washer, reader, computer, printer) is required.

Beyond these, as optional for now, but necessary in few years time, the following equipment is required:

- one ultracentrifuge
- one Eppendorf centrifuge
- one Polymerise Chain Reaction (PCR) machine
- apparatus for nucleic acid and protein electrophoresis

Personnel

The personnel should at least consist of one competent veterinarian and two experienced assistants.

COUNTRY REPORTS ON AVIAN INFLUENZA AND NEWCASTLE DISEASE FOR 2000 BASED ON RESPONSES TO THE QUESTIONNAIRE

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INTRODUCTION

As a substitute for the "country reports" presented at previous meetings, which had proved increasingly time consuming, it was decided for the present 7th meeting to present a summary report. The information for this report was to be taken from answers supplied by National laboratories to the following questionnaire:

NEWCASTLE DISEASE & AVIAN PARAMYXOVIRUSES

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

Example response:

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

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

Example response:

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

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

Example response:

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

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

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

Example response:

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

AVIAN INFLUENZA

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

Example response:

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

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

Example response:

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

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

Example response:

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

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

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

A total of 29 questionnaires was sent to different laboratories. Responses were received for 13 laboratories of EU countries and 7 from non-EU countries. Although data was requested for 1999 and 2000 many countries replied only for 2000 and this report is restricted to that year. The responses are summarised in the following pages.

BELGIUM/LUXEMBOURG

Samples tested

Type of bird	Sample	Method	Number
pigeons	tissues	eggs	49
	tissues	cell culture	49
	swabs	eggs	2
	swabs	cell culture	2
layers	tissues	eggs	65
	tissues	cell culture	65
psittacines	tissues	eggs	22
	tissues	cell culture	22
backyards	tissues	eggs	13
	tissues	cell culture	13
other birds	tissues	eggs	7
	tissues	cell culture	7
ducks and geese	tissues	eggs	2
	tissues	cell culture	2

Paramyxoviruses isolated

Type of bird	isolates
pigeons	10x PPMV-1
layers	3 x APMV-1 La Sota type
	1 x APMV-1 Hitchner type
psittacines	2 x APMV not 1

Characterisation of APMV-1 viruses isolated

Bird	ICPI	amino acids at cleavage site	mAb group	conclusion
Pigeons		^{112}RRQKRF^{117}	P	PPMV-1
Layers			La Sota type	Vaccine
Layers			Hitchner type	Vaccine

Influenza viruses isolated

None.

BULGARIA

During 2000 there were six investigations of Newcastle disease in poultry no virus was isolated.

100 waterfowl were tested for influenza – all tests were negative.

CYPRUS

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
parrots/canaries	tissues	16
wild birds	tissues	2

Paramyxoviruses isolated

parrots/canaries 3 x APMV-3 [psittacine group]

Influenza viruses isolated

None

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
Ostriches	264	AGP	negative
Turkeys	80	AGP	negative

CZECH REPUBLIC

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
pigeons	tissues	14
broilers	tissues	3

Paramyxoviruses isolated

pigeons 6 x APMV-1

Characterisation of AMPV-1 isolates

Bird	**ICPI**	**RT-PCR**	**Conclusion**
pigeon	0.53	nd	vaccine
pigeon	0.58	nd	vaccine
pigeon	0.52	low virulence	vaccine
pigeon	0.16	low virulence	vaccine
pigeon	0.67	low virulence	vaccine
pigeon	0.55	low virulence	vaccine

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

None.

DENMARK

Samples tested by inoculation into eggs or cell cultures

Type of bird	Sample/method	Number
Chickens	tissues/eggs	522
Chickens	cloacal swabs/eggs	26
Chickens	tracheal swabs/eggs	8
Chickens	tissues/cells	10
Turkeys	tissues/eggs	36
Turkeys	tracheal swabs/eggs	6
Ostriches	tissues/eggs	40
Pigeons	tissues/eggs	131
Pheasants	tissues/eggs	243
Pheasants	cloacal swabs/eggs	6
Pheasants	tracheal swabs/eggs	6
Geese	tissues/eggs	15
Ducks	cloacal swabs/eggs	6
Ducks	tracheal swabs/eggs	6
'Waterfowl'	tissues/eggs	12
Parrots	tissues/eggs	176
Other pet birds	tissues/eggs	103
Zoo birds	tissues/eggs	104
Other birds	tissues/eggs	43

Wild bird surveys

During 2000 surveillance of wild birds resulted testing in cloacal swabs from 40 moorhens (*Gallinula chloropus*), 85 teals (*Anas crecca*), 20 mallards (*Anas platyrhynchos*) and 10 snipes (*Gallinago gallinago*).

Paramyxoviruses isolated

Pigeons 10 x APMV-1 [PPMV-1]
Pheasants 2 x APMV-1
Ducks 1 x APMV-1

Characterisation of AMPV-1 isolates

Bird	ICPI	Amino acids at cleavage site	MAb group	Conclusion
Pigeons	ND	ND	P	PPMV-1
Ducks	0.3	^{112}GKQGRL^{117}	(?)	Low virulence PMV-1
Pigeons	ND	ND	P	PPMV-1
Pigeons	ND	ND	P	PPMV-1
Pigeons	ND	ND	(P)	PPMV-1
Pigeons	ND	ND	(P)	PPMV-1
Pheasants	1.69	^{112}RRQRRF^{117}	C1	Virulent PMV-1 in free-living birds
Pheasants	1.66	^{112}RRQRRF^{117}	(C1)	Virulent PMV-1 in free-living birds
Pigeons	ND	ND	(P)	PPMV-1
Pigeons	ND	ND	(P)	PPMV-1
Pigeons	ND	ND	(P)	PPMV-1
Pigeons	ND	ND	(P)	PPMV-1
Pigeons	ND	ND	(P)	PPMV-1

(X) = Awaiting conformation, ND = Not determined yet.

Influenza viruses isolated

From the surveillance exercise.
Teals (*Anas crecca*) 1 x H3
Mallards (*Anas platyrhynchos*) 1 x H6, 1 x H11(?)

Serological monitoring for APMV-1

Type of birds	No. of samples
Chickens	6795
Waterfowl	85
Ostriches	4
Free living birds	267
Pigeons	1
Other birds	36

ESTONIA

Samples tested

Type of bird	Sample	Method	Number
broilers, egg layers	tissues	eggs	42

Paramyxoviruses isolated

None.

Influenza viruses isolated

None.

Serological monitoring for APMV-1

Broilers and egg layers were tested

Number of flocks tested	Number of sera examined	Number of flocks positive	Number of sera positive	Test
17	2306	4	792	ELISA
8	266	-	-	HI

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

None.

FINLAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Chickens	tissues/cloacal swabs	51
Turkeys	tissues/cloacal swabs	4
Geese	tissues/cloacal swabs	6
Ducks	tissues/cloacal swabs	37
Pigeons	tissues/cloacal swabs	4
Wild birds	tissues/cloacal swabs	32
Caged birds	tissues/cloacal swabs	7

Paramyxoviruses isolated

None.

Serological monitoring for APMV-1

Type of poultry	No. flocks tested	No. sera examined	No. flocks positive	No. sera positive
Layers	42	1604	1 (hobby flock)	1
Broiler	101	5156	0	0
Turkey	52	1513	1	18
Duck	2	67	1	4
Geese	1	23	1	2
Ostrich	4	9	0	0

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

None.

GERMANY

Samples tested by inoculation into eggs

Species	processed samples	number of isolates*	submitted to National RL
Chicken	806	30	12
Turkey	62	0	0
Duck	76	0	0
Goose	50	1	0
Ornamental poultry	49	1	1
Pigeon	549	81	52
Ostrich	7	0	0
Parakeet/parrot	218	8	8
Ornamental birds (small)	61	3	2
Capercaillie (Import)	27	7	3
Wild birds (small)	202	0	0
Crow/raven	23	2	0
Bird of prey/owl	67	0	0
Crane	6	0	0
Stork	7	0	0
Großtrappe	2	0	0

*all isolates were APMV-1

Characterisation of AMPV-1 isolates

Bird	number	ICPI	cleavage site	mAb	conclusion
Chickens	11 1		GRQGRL GRQGRL	E 7D4-ve	vaccine
Ornamental poultry	1			P	PPMV-1
Pigeons	50 2		RRKKRF GRQGRL	P E	PPMV-1 vaccine
Parakeets/parrots	1 5		GRQGRL ND	E P	vaccine PPMV-1
Parakeets in quarantine	2	1.7	RRQKRF		virulent APMV-1
Ornamental birds	2			P	PPMV-1
Capercaillie in quarantine	3	1.2-1.3	RRKRF	P	PPMV-1

Influenza viruses isolated

none

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	No. positive	Subtype
Chicken	13,040	9	H1
Turkeys	22,104	110 45	H6 H1
Ducks	73	0	
Geese	227	0	
Ostriches	40	0	
Pigeons	18	0	
Gulls	27	0	

5 samples from chickens and 9 samples from turkeys were suspect for H7 antibodies but on re-sampling the flocks all samples were negative.

GREECE – AVIAN PARAMYXOVIRUS LABORATORY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Broilers	tissues	25
Partridge	tissues	8
Pigeons	tissues	10

Paramyxoviruses isolated

None

Influenza viruses isolated

None

Serology for APMV-1

In Greece vaccination is voluntary.

Type of birds	No. of samples	Method used	Result
broilers and breeder	141	HI	Positive vaccinated

Serological monitoring for avian influenza antibodies

1268 sera samples from broilers were examined and found negative in the year 2000. The broilers were imported from Italy.

GREECE – AVIAN INFLUENZA LABORATORY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
chickens	faeces	210
wild birds	tissues	34

Paramyxoviruses isolated

None

Influenza viruses isolated

None

Serological monitoring for avian influenza antibodies

Type of birds	No. of samples	Method used	Result
chicks imported from Italy	2580	AGP	negative
layers, breeders and turkeys	700	AGP	negative

HUNGARY

Samples tested

Type of bird	Sample	Method	Number
broiler chickens	tissues	eggs/cell culture	5
geese	tissues	eggs/cell culture	37
turkeys	tissues	eggs/cell culture	1

Paramyxoviruses isolated

None.

Influenza viruses isolated

None.

Influenza virus serology

Type of bird	Number tested	Test	Results
Turkeys	6	AGID	Negative

IRELAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Broilers	cloacal/tracheal swabs	25
Broilers	tissues	34
Turkeys	tissues	2
Pheasants	tissues	2
Geese	tissues	2
Penguins	tissues	2
Pigeons	tissues	4
Canary	tissues	1
Ornamental fowl	tissues	2

Paramyxoviruses isolated

ducks 1 x APMV-1
pigeons 2 x APMV-1

Characterisation of APMV-1 isolates

Bird	ICPI	amino acids	mAb group	conclusion
duck	0		H	vaccine
pigeon			P	PPMV-1
pigeon			P	PPMV-1

Influenza viruses isolated

None.

ITALY

Samples tested in eggs:

Type of bird	Sample	Number
Broiler breeders	tissues	12
Broiler breeders	pools of cloacal swabs	121
Broiler breeders	pools of faeces	4
Layer breeders	tissues	2
Layer breeders	pools of cloacal swabs	24
Layer breeders	pools of faeces	1
Broilers	tissues	88
Broilers	pools of cloacal swabs	54
Broilers	pools of faeces	7
Layers	tissues	42
Layers	pools of cloacal swabs	56
Layers	pools of faeces	10
Rural chickens	tissues	93
Turkey breeders	tissues	4
Turkey breeders	pools of cloacal swabs	78
Meat turkeys	tissues	313
Meat turkeys	pools of cloacal swabs	57
Meat turkeys	pools of faeces	21
Meat turkeys	pools of tracheal swabs	3
Pheasants	tissues	7
Pheasants	pools of cloacal swabs	10
Quail	tissues	7
Quail	pools of cloacal swabs	5
Guinea fowl	tissues	21
Guinea fowl	pools of cloacal swabs	17
Ostriches	tissues	6
Ostriches	pools of cloacal swabs	3
Ostriches	pools of faeces	5
Geese	tissues	3
Ducks	tissues	
Ducks	pools of cloacal swabs	7
Ducks	pools of faeces	1
Pigeon	tissues	307
Pigeons	pools of cloacal swabs	4
Ducks	pools of faeces	1
Collared doves	tissues	25
Sparrows	tissues	2
Wild birds	pools of cloacal swabs	2
African passerine (imported)	faeces	2

Paramyxoviruses isolated

Type of bird	Viruses
Broiler breeders	1x APMV-1
Layer breeders	1x APMV-1
Broilers	19 x APMV-1
Broilers	1 x PPMV-1
Layers	4 x APMV-1
Layers	1 x PPMV-1
Rural chickens	87 x APMV-1
Meat turkey	8 x APMV-1
Pheasant	6 x APMV-1
Quail	1 x APMV-1
Guinea fowl	3 x APMV-1
Ostrich	1 x APMV-1
Pigeon	16 x PPMV-1
Collared dove	6 x PPMV-1
African passerine (imported)	2 x APMV-2

Characterisation of APMV-1 isolates

Bird	***ICPI*** [range]	Amino acids	mAb group	Conclusion
Broiler breeders	not done	^{112}RRQRRF^{117}	C1	Newcastle disease
Layer breeders	not done	^{112}RRQRRF^{117}	C1	Newcastle disease
Broilers	0.2-2	^{112}RRQRRF^{117} ^{112}GRQGRL^{117} ^{112}RRQRRF^{117}	P E C1	1 x PPMV-1 2 x Vaccine 17 x Newcastle disease
Layers	0.9- 1.9	^{112}GRQKRF^{117} ^{112}RRQRRF^{117}	P C1	1xPPMV-1 4 x Newcastle disease
Rural chicken	0.2- 2	^{112}GRQGRL^{117} ^{112}RRQRRF^{117}	E C1	1x Vaccine 86 x Newcastle disease
Meat turkey	1.6-1.8	^{112}RRQRRF^{117}	C1	8 x Newcastle disease
Pheasant	0.1-1.8	not done	E C1	1x Vaccine 5 x Newcastle disease
Guinea fowl	not done	^{112}RRQRRF^{117}	C1	3 x Newcastle disease
Ostrich	1.8	^{112}RRQRRF^{117}	C1	1 x Newcastle disease
Quail	1.8	not done	C1	1 x Newcastle disease
Pigeon	0.5 -1.7	1 x ^{112}RRKKRF^{117} 1 x ^{112}GRQKRF^{117}	P P	16 x PPMV-1
Collared dove	0.68-1.28	1 x ^{112}RRKKRF^{117}	P	6 x PPMV-1

Influenza viruses isolated

Avian influenza viruses of H7N1 subtype were isolated from the following:

Type of bird	No. isolates
Broiler breeders	6
Broilers	14
Layers	20
Rural chickens	6
Turkey breeders	3
Meat turkey	134
Pheasant	1
Quail	1
Guinea fowl	3
Ostrich	3
Goose	1
Collared dove	1
Sparrow	2

Characterisation of AI viruses

Birds	Subtype	IVPI	HA0 cleavage site	Conclusion
Broiler breeders	H7N1	3	***PEIPKGSRVRR*GLF***	6 x HPAI
Broilers	H7N1	3	PEIPKGSRVRR*GLF	14 x HPAI
Layers	H7N1	3	PEIPKGSRVRR*GLF	20 x HPAI
Rural chickens	H7N1	3	PEIPKGSRVRR*GLF	6 x HPAI
Layer breeders	H7N1	3	PEIPKGSRVRR*GLF	1x HPAI
Turkey breeders	***H7N1***	3	PEIPKGSRVRR*GLF	3 x HPAI
Meat turkey	H7N1	0.00 - 3	PEIPKGSRVRR*GLF PEIPKGR*GLF	99x HPAI 35x LPAI
Pheasant	H7N1	3	PEIPKGSRVRR*GLF	1x HPAI
Quail	H7N1	3	PEIPKGSRVRR*GLF	1x HPAI
Guinea fowl	H7N1	3	PEIPKGSRVRR*GLF	3 x HPAI
Ostriches	H7N1	3	PEIPKGSRVRR*GLF	3 x HPAI
Goose	H7N1	3	PEIPKGSRVRR*GLF	1x HPAI
Collared dove	H7N1	3	PEIPKGSRVRR*GLF	1x HPAI
Sparrows	H7N1	3	PEIPKGSRVRR*GLF	2 x HPAI

NORWAY

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Broiler breeders	cloacal swabs	202
Broiler breeders	tissues	36

Paramyxoviruses isolated

3 x APMV-1

Characterisation of APMV-1 isolates

Bird	ICPI	Amino acids	mAb group	Conclusion
Broiler breeders	0.00	^{112}GKQGRL^{117}	?	low virulence APMV-1

Serological monitoring for APMV-1

Type of poultry	No. flocks tested	No. sera examined	No. flocks positive	No. sera positive
For APMV-1				
Fowl	174	8704	4	404
Turkey	14	715	0	0
Duck	5	241	0	0
Geese	3	141	0	0
For APMV-3				
Fowl	2	59	0	0
Turkeys	2	48	2	26

Influenza viruses isolated

None.

Influenza virus serology

Type of bird	No. of flocks	No. of sera	Test	Results
Turkeys	2 [imported]	117	HI for H5 and H7	Negative
Fowl	14 [imported]	710	HI for H5 and H7	Negative

PORTUGAL

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
pigeons	tissues	2

Paramyxoviruses isolated

pigeons 2 x APMV-1

Characterisation of AMPV-1 isolates

Bird	ICPI	Amino acids at cleavage site	MAb group	Conclusion
Pigeons	ND	ND	P	PPMV-1
Pigeons	ND	RRQKRF	P	PPMV-1

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

None.

SLOVENIA 1999/2000

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Broilers	tissues	28
Broiler breeders	tissues	7
Pigeons	tissues	8
Meat turkeys	tissues	6

Paramyxoviruses isolated

Pigeons 3x APMV-1

Characterisation of AMPV-1 isolates

Bird	ICPI	Amino acids*	MAb group	Conclusion
Wild pigeon	1.1	^{112}GRQKRF^{117}	P	PPMV-1
Wild pigeon	0.9	^{112}GRQKRF^{117}	P	PPMV-1
pigeon	0.9	^{112}GRQKRF^{117}	P	PPMV-1

N.D. not done; *samples were sent to VLA, Weybridge

Influenza viruses isolated

None

Serological monitoring for avian influenza antibodies

Type of birds	No of samples/flocks	Method used	Result
Meat turkeys	80/5	AGP	Negative
Meat turkeys	225/6	ELISA	Negative
Broilers	59/3	ELISA	Negative
Broiler breeders	70/2	ELISA	Negative

SPAIN

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Duck	tissues	1
Eagle	tissues	3
Ostrich	cloacal swabs	9
Owl	tissues	6
Partridge	tissues	15
Pigeon	tissues	7
Stork	tissues	1

Paramyxoviruses isolated

None.

Serological monitoring for APMV-1

Type of bird	No. sera examined	No. sera positive
Amazonia esquivia	3	0
Canary	4	0
Duck	1	0
Eagle	3	0
Owl	6	0
Ostrich	220	0
Parrot	21	0
Partridge	15	0
Stork	1	0

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

Type of bird	No. sera examined	No. sera positive
Amazonia esquivia	3	0
Canary	4	0
Duck	1	0
Eagle	3	0
Ostrich	638	0
Owl	6	0
Parrot	3	0
Partridge	15	0
Stork	1	0

SWEDEN

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Broilers	tissues cloacal swabs	101 300
Pigeons	tissues	10
Wild birds	tissues	159

Paramyxoviruses isolated

pigeons 5 x APMV-1

Characterisation of AMPV-1 isolates

Bird	ICPI	Amino acids at cleavage site	MAb group	Conclusion
Pigeons	1.1	ND	P	PPMV-1
Pigeons	0.72	ND	P	PPMV-1
Pigeons	0.87	ND	P	PPMV-1
Pigeons	ND	ND	P	PPMV-1
Pigeons	0.8	ND	P	PPMV-1

Serological monitoring for APMV-1

Type of bird	No. flocks tested	No. sera examined	No. flocks positive	No. sera positive
Breeders	116	6699	0	0
Imported breeders in quarantine	22	1573	2	391
Turkeys	21	1279	0	0
Pigeons		3	0	3
wild birds		182	0	1
Back yard flocks	42	927	0	0

Influenza viruses isolated

None.

Serological monitoring for avian influenza antibodies

Type of poultry	No. flocks tested	No. sera examined	No. flocks positive	No. sera positive
Breeders	72	4238	0	0
Imported breeders in quarantine	13	260	0	0
Turkeys	10	400	0	0

UK - GREAT BRITAIN

Samples tested

Type of bird	Sample	Method	Number
Chickens	tissues/cloacal swabs	eggs	228
Turkeys	tissues/cloacal swabs	eggs	134
Game birds	tissues/cloacal swabs	eggs	78
Pigeons	tissues/cloacal swabs	eggs/cell culture	55
Waterfowl	tissues/cloacal swabs	eggs/cell culture	59
Caged birds in quarantine	tissues/cloacal swabs	eggs	31
Other birds	tissues/cloacal swabs	eggs	15

Paramyxoviruses isolated

Type of bird	Isolates
pigeons	26 x PPMV-1
caged birds	2 x APMV-2

Characterisation of AMPV-1 isolates

Bird	ICPI	MAb group	Conclusion
Pigeons	ND	26 x P	26 x PPMV-1

Influenza viruses isolated

Bird	subtype	IVPI	conclusion
caged birds	H3N8	0.00	LPAI

Influenza serology

Testing of birds for export by haemagglutination inhibition tests for H5 and H7 antibodies

Type of bird	Number tested	Results
Ducks	9300	all negative
Chickens	105	all negative
Turkeys	40	all negative

UK - NORTHERN IRELAND

Samples tested by inoculation into eggs:

Type of bird	Sample	Number
Chickens	cloacal swabs	16
Chickens	tissues	100
Pigeons	tissues	34
Pheasants	tissues	4
Turkeys	tissues	2

Paramyxoviruses isolated

Type of bird	No. isolates
Broilers	3 x APMV-1
Pigeons	4 x APMV-1

Characterisation of AMPV-1 isolates

Bird	ICPI	amino acids	mAb group	Conclusion
Broiler	0.00	-	E	vaccine
Broiler	0.00	-	E	vaccine
Broiler	0.00	-	E	vaccine
Pigeon	0.96	^{112}RRKKRF^{117}	P	PPMV-1
Pigeon	1.26	-	B	?novel pigeon isolate
Pigeon	0.74	-	B	?novel pigeon isolate
Pigeon	1.03	-	B	?novel pigeon isolate

Influenza viruses isolated

None

Influenza serology

Type of bird	No. Tested	Positive	Negative	Positive	**Negative**
		H5(N3)		H7(N7)	
Chicken	108	0	108	2[b]	106
Exotic	73	1[a]	72	1[a]	72
Pheasant	16	0	16	1[c]	15
TOTAL	197	1	196	4	193

[a]Post-import test on zoo pelican, [b]Tissues negative for AIV by virus isolation in eggs, [c]Mortalities in birds belonging to a gun club; tissues negative for AIV by virus isolation in eggs, *Erysipelothrix rhusiopathiae* septicaemia diagnosed.

DISCUSSION

The responses to the questionnaires show the wide disparity for testing for ND and AI in the 20 countries responding. In the majority surveillance is 'passive' i.e. responding only to disease investigations and trade or vaccine status requirements. Some more active surveillance appears to have been carried out in Denmark, Sweden and Germany. The problems of HPAI and ND in Italy during 2000 resulted in a huge numbers of samples being tested for diagnostic purposes and this overshadowed all other activities in the countries replying (details of the Italian outbreaks appear elsewhere in these proceeding).

Infections in pigeons with the variant PPMV-1 still seems to be common and widespread in Europe. In total 129 isolates of PPMV-1 were made from pigeons or doves in 9 countries [Belgium 10, Denmark 10, Germany 50, Ireland 2, Italy 22 (including 6 from feral collared doves), Slovenia 3, Sweden 5, Great Britain 26 and Northern Ireland 1]. In addition 3 APMV-1 isolates from pigeons in Northern Ireland appeared to be PPMV-1 viruses but showed an unusual mAb binding pattern. PPMV-1 viruses were also reported from ornamental poultry [1 isolate], ornamental birds, [2 isolates], psittacines [5 isolates] and capercaillie in quarantine [3 isolates] by Germany and broilers [1 isolate] and layers [1 isolate] in Italy, emphasising the potential of this virus to spread to other birds. Apart from Italy, the only other reported outbreaks of virulent APMV-1 viruses were the two from pheasants in Denmark, with ICPI values of 1.66 and 1.69 and showing a C1 mAb binding pattern, and 2 isolates [ICPI 1.7] from parakeets in quarantine in Germany.

Only 3/20 countries reported isolations of influenza viruses. Apart from the HPAI and LPAI H7N1 isolates from the widespread outbreaks in Italy these were as follows: In Denmark three viruses of H3, H6 and H11? subtypes were isolated from ducks during their surveillance of wild birds. In Great Britain a single isolate of H3N8 subtype was obtained from birds in quarantine.

A number of laboratories reported various levels of serological testing for influenza virus infections. In Germany 9/13,040 chicken sera were positive for H1 antibodies, 110/22,104 turkey sera positive for H6 antibodies and 45/22,104 turkey sera positive for H1 antibodies. In N. Ireland 2/108 chicken and 1/15 pheasant sera tested were positive for H7 antibodies although no virus could be isolated in either case. An imported zoo pelican had positive titres for both H5 and H7 subtypes. None of the six other laboratories reporting some degree of serology for influenza recorded any positive results.

The results presented in this paper suggest that the H7N1 influenza viruses causing such problems in Italy during 2000 did not spread to other countries and the prevalence of influenza infections remains very low in poultry outside Italy. The apparent prevalence of classical virulent ND also remains extremely low outside Italy, but there is widespread use of vaccine and a lack of surveillance of vaccinated birds. The variant ND virus termed PPMV-1 continues to circulate and present a threat poultry and other birds.

COMPARATIVE TESTS FOR ANTIGEN IDENTIFICATION IN DIFFERENT NATIONAL LABORATORIES 2000

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INTRODUCTION

One of the functions and duties of the Community Reference Laboratories for Newcastle Disease and Avian Influenza is to organise "periodical comparative tests in diagnostic procedures at Community level". To fulfil this duty a simple test of the ability of the National Laboratories to identify Newcastle disease and influenza was organised in 1998 (Alexander and Manvell, 1999). Although the results of this test were not disastrous there were sufficient areas of concern and as a result the following recommendations were made and similar tests repeated for 1999. There was relatively little improvement (Alexander and Manvell, 2000) and it was decided at the Sixth Annual Meeting to repeat antigen identification in 2000. Again the objectives of the test were to be:

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

In the past results have been kept confidential to the submitting laboratory. However, it was suggested that this was unnecessary by representatives of several participating laboratories. Prior to the meeting 29 potential participating laboratories were asked whether or not they wished the results of the comparative tests obtained by each laboratory to be identified with each laboratory. Eighteen laboratories answered; 6 replied yes [the laboratories should be identified], 12 replied no [the individual results should remain confidential to the participating laboratory]. As a result it was decided to retain anonymity.

MATERIALS AND METHODS

Each National Laboratory was sent 7 unknown antigens with instructions to carry out identification of the antigens A-G by HA and HI tests.

The antigens supplied were formalin or betapropiolactone inactivated whole viruses. Laboratories are expected to be at least able to identify H5 and H7 influenza viruses and APMV-1 [Newcastle disease] virus. However implicit in this expectancy is that they will not erroneously identify other viruses as these. The antigens supplied were therefore selected to test these points. It was not necessarily expected that every National Laboratory would fully identify all the antigens, but should be able to reach the minimum acceptable standard.

The antigens supplied and the minimum essential results were:-

Antigen	Virus	Minimum essential result
A	APMV-1/chicken/Ulster/2C/67	APMV-1
B	APMV-1/chicken/Ulster/2C/67	APMV-1
C	PPMV-1/pigeon/England/617/83	APMV-1
D	APMV-3/turkey/England/1087/82	APMV-3
E	A/African starling/EnglandQ/983/79 (H7N1)	H7
F	A/ostrich/Denmark/72420/96 (H5N2)	H5
G	A/duck/Alberta/35/76 (H1N1)	other

RESULTS

General

Twenty-six of the 29 laboratories that had been sent samples responded by submitting results. These results are shown in Table 1 for EU Member States and Table 2 for Non-EU countries. All 16 EU laboratories responded, this included additional laboratories for N. Ireland and separate influenza and Newcastle disease laboratories for Greece. While Belgium acts as both reference laboratories for Luxembourg. Laboratories from 10 non-EU states participated these were: Bulgaria, Cyprus, Czech Republic, Estonia, Hungary, Lithuania, Norway, Poland, Slovenia and Switzerland. One country, code 13, had clearly suffered some serious problem in carrying out the tests and was omitted from further analyses of the results.

In total 173 results were received from the 25 laboratories. The correct results were obtained on 141 [81.4%] occasions. Results judged not to be wholly correct without actually being wrong [lesser-shaded cells in Tables 1 and 2] were given on 18 [10.4%] occasions. Fourteen [8.1%] were wrong either because they failed to identify APMV-1, H5 or H7 antigens, or because they identified the H1N1 virus as H7 [see 14 G and 23 G in Table 1]

Of the 25 participating laboratories, 10 fully identified all HA antigens and a further 5 obtained at least the minimum results. Six laboratories had one unacceptable result and 4 had more than one unacceptable result.

Results by antigen

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

APMV-1/chicken/Ulster/2C/67 is the virus recommended for use as the standard antigen in haemagglutination inhibition tests in the EU, identification as APMV-1 should therefore have been straightforward. Two laboratories [23 and 9] failed to give the correct result [23 with some mitigating circumstances] both identifying the antigen as an APMV virus, but not specifically APMV-1.

ANTIGEN B – virus APMV-1/chicken/Ulster/2C/67 – correct result APMV-1
Antigen B was a duplication of Antigen A being a vial from the same freeze-dried batch. One laboratory failed to detect any haemagglutination in the re-suspended vial contents. Of the other 24, three laboratories [23, with some mitigating circumstances, 5 and 9] failed to produce the correct result.

ANTIGEN C – virus PPMV-1/pigeon/England/617/83 – correct result PPMV-1 [minimum acceptable result APMV-1]

There is a monoclonal antibody [mAb], 617/161, available from the Community Reference Laboratory that reacts in HI tests with the vast majority of isolates of the variant "pigeon" strain of APMV-1, but not other APMV-1 viruses. There is no reason why this mAb should not have been used to fully identify this antigen.

Sixteen laboratories fully identified antigen C as PPMV-1 and a further 5 gave the correct result of APMV-1. The four other laboratories gave the incorrect results of APMV-3 [10], APMV-7? [2], APMV-? [24] and "other" [17].

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

Although it is theoretically sufficient to identify APMV-3 viruses as 'not APMV-1', because some of these show such high level of cross relationship with APMV-1 viruses it is essential for reliable diagnosis that they are fully identified. Further it was one of the recommendations made in the proceedings of the 5th Annual Meeting [Alexander and Manvell, 1999] that all laboratories should hold APMV-3 antiserum to enable identification. One laboratory [5] reported no HA activity in the re-constituted vial. The 3 laboratories that failed to identify antigen C as APMV-3 [23, 10, 25] were unable to specify the APMV subtype.

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

Identification of the antigen as an H7 influenza virus should have been straightforward. However, one EU laboratory [17 – with mitigating circumstances] and two non-EU laboratories [5 and 25] failed to identify the antigen.

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

The same three laboratories failed to identify this antigen. One laboratory [25] identified it as H9N2, this is probably due to the use of an H9N2 antiserum that gave positive results in the HI test due to the shared N2 subtype. Although not necessary for diagnosis or the purposes of this exercise, six laboratories gave the additional information of the N subtype, unfortunately one laboratory [3] characterised this as N3 instead of N2.

ANTIGEN G – virus A/duck/Alberta/35/76 (H1N1) - correct result not APMV-1, H5 or H7 – preferred result H1

Two laboratories failed to submit results for this antigen. Of the remaining 23, 18 gave the correct result of not H5, H7 or APMV-1, 10/18 identifying the virus as H1, the preferred result. Three laboratories gave the result as other or ?, but two laboratories [14 and 23] gave the wholly wrong result of H7.

DISCUSSION

One of the objectives of the comparative tests is that laboratories should be able to take remedial measures where they have fallen short of the desired standard. Of the laboratories taking part in 2000 21 had taken part in 1999. The comparative results for the two years were:

Number that:-

	1999	2000
Fully identified all antigens:	6	16
Obtained at least minimum	5	0
Had one unacceptable result	7	3
Had more than one wrong	3	2

In fact 11 laboratories showed an improvement; six were the same, in this case all results were correct; 4 laboratories obtained worse results than 1999. No country fell into any other possible category.

Despite these improvements there is still a worrying number of unacceptable results and further comparative tests are recommended during 2002.

REFERENCES

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TABLE 1:

RESULTS OBTAINED IN COMPARATIVE ANTIGEN IDENTIFICATION TESTS 2000 – EUROPEAN UNION MEMBER COUNTRIES

Code	A	B	C	D	E	F	G
0	NDV Ulster 2C	NDV Ulster 2C	PPMV-1	APMV-3	H7N1	H5N2	H1N1
1*	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	H1
3	APMV-1	APMV-1	PPMV-1	APMV-3	H7N1	H5N3	H1N2
4	APMV-1	APMV-1	PPMV-1	APMV-3	H7[N1?]	H5[N2?]	H1
6	APMV-1	APMV-1	PPMV-1	APMV-3	H7N1	H5N2	H not 5 or 7 N1
7	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	H1
8	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	not done
11	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	not H5, H7 or NDV
12	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	H1
14	APMV-1	APMV-1	PPMV-1	APMV-3	H7N1	H5N2	**H7[N2?]**
16	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	H1? not H5 or H7
17	APMV-1	APMV-1	**other**	APMV-3	other	other	other
19	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	H1
20	APMV-1	APMV-1	PPMV-1	APMV-3	H7[N1?]	H5N2	H1N1
21	APMV-1	APMV-1	APMV-1	APMV-3	H7	H5	H1
23	APMV-1/3	APMV-1/3	APMV-1/3	APMV-1/3	H7	H5	**H7**
26	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	flu not H5 or H7

*The laboratory codes used in Tables 1 and 2 represent the chronological order that results were received.

TABLE 2:

**RESULTS OBTAINED IN COMPARATIVE ANTIGEN IDENTIFICATION TESTS 2000–
NON-EUROPEAN UNION MEMBER COUNTRIES**

Code	A	B	C	D	E	F	G
2*	APMV-1	APMV-1	**APMV-7?**	APMV-3	H7[N1?]	H5[N2?]	flu not H5 or H7
5	APMV-1	**?**	APMV-1	no HA	**?**	**?**	?
9	**APMV-?**	**?**	APMV-1	APMV-3	H7	H5	flu
10	APMV-1	APMV-1	**APMV-3**	APMV-?	H7	H5	flu
13							
15	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	flu
18	APMV-1	APMV-1	PPMV-1	APMV-3	H7	H5	flu not H5 or H7
22	APMV-1	APMV-1	APMV-1	APMV-3	H7	H5	H1
24	APMV-1	APMV-1	**APMV-?**	APMV-3	H7	H5	not done
25	APMV-1	no HA	APMV-1	**APMV-1/3**	**other**	**H9N2**	other

*The laboratory codes used in Tables 1 and 2 represent the chronological order that results were received.

PAPERS PRESENTED AS ORIGINAL CONTRIBUTIONS

CLINICAL, GROSS AND MICROSCOPIC FINDINGS IN DIFFERENT AVIAN SPECIES NATURALLY INFECTED DURING THE H7N1 HIGHLY PATHOGENIC AVIAN INFLUENZA AND NEWCASTLE DISEASE EPIDEMICS IN ITALY DURING 1999 AND 2000

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INTRODUCTION

Avian influenza viruses may be classified on the basis of the clinical condition they determine in susceptible birds. Low pathogenicity avian influenza (LPAI), may be caused by viruses belonging to all 15 haemagglutinin types (H1-H15) and determine a mild disease in susceptible poultry, characterised by respiratory and enteric signs that are often associated in breeders and table-egg layers to reproductive abnormalities. Highly pathogenic avian influenza (HPAI), that is caused by only certain viruses of the H5 and H7 subtypes, is, instead a devastating disease of poultry with mortality rates which approach 100% in gallinaceous birds.

Evidence collected from recent influenza outbreaks indicate that LPAI viruses belonging to the H5 and H7 subtypes, may mutate and become HPAI, probably after introduction to poultry (Garcia *et al.,* 1996, Perdue *et al.,* 1997) resulting in extremely complex situations which may have dramatic effects on the poultry industry.

Italy has been affected by both HPAI and LPAI throughout the years. However, in recent years, a minor epidemic of HPAI, caused by a virus of the H5N2 subtype in semi-intensive and backyard farms in 1997-1998 occurred in north-eastern Italy (Capua *et al.,* 1999b) and a limited number of isolations LPAI have been recorded from 1990 (Papparella *et al.,* 1994; Papparella *et al.*, 1995).

Newcastle disease (ND) is a viral infection of birds caused by an avian paramyxovirus serotype 1 (APMV-1), that, together with other eight APMV serotypes, has been placed in the genus *Rubulavirus*, sub-family *Paramyxovirinae*, family *Paramyxoviridae*, Order *Mononegavirales* in the current taxonomy (Rima *et al*., 1995). ND is a highly contagious and diffusive disease and can cause a very severe condition in susceptible birds, with mortality rates exceeding 50% in chickens.

Both HPAI and ND are included in List A of the Office International des Epizooties (OIE), and statutory measures for their control are implemented in the European Union (CEC, 1992a; CEC, 1992b).

During 1999 north eastern Italy has been affected by an epidemic of LPAI due to a virus of the H7N1 subtype. The epidemic involved 199 outbreaks and caused considerable losses to the poultry industry (Capua *et al.,* 1999b). In the month of December 1999 the H7N1 LPAI virus mutated to a HPAI virus, which rapidly spread, causing 413 outbreaks, and determining direct or indirect death of over 14 million birds of different species (Capua & Marangon, 2000; Capua *et al*., 2000a). Following the HPAI epidemic, Newcastle Disease (ND) was introduced in Italy (Capua *et al.*, present meeting), and affected a number of industrial establishments, of semi intensive and backyard flocks.

In the present paper we report on the clinical, gross and microscopic findings recorded in affected birds during the HPAI and ND epidemics.

MATERIALS AND METHODS

Laboratory investigations. Birds of different species exhibiting clinical signs were submitted for laboratory investigations including post-mortem examination, bacteriology, histopathology and attempted virus isolation.
Following the implementation of Directives 92/40/EEC and 92/66/EEC (CEC, 1992a; CEC, 1992b), samples were collected from all infected and suspect flocks for virological investigation. Virological investigations were performed in accordance with the guidelines reported in the above mentioned directives. Avian influenza isolates were characterised as reported by Alexander and Spackman (1981). The virulence of the avian influenza isolates was determined through the intravenous pathogenicity index test [IVPI] (CEC, 1992a) and by nucleotide sequencing in the region of the genome coding for the cleavage site of the haemagglutinin molecule (Wood *et al.,* 1994; Wood *et al.,* 1997). The virulence of ND isolates was established by means of the intracerebral pathogenicity index test [ICPI], and by sequencing the genome segment which encodes for the cleavage site of the F protein.

Selected organs were sampled and immediately fixed in 10% phosphate-buffered formalin. Tissues were embedded in paraffin, sectioned at 3 μm and stained with hematoxylin & eosin. Unstained paraffin embedded sections were immunohistochemically examined for presence of influenza A nucleoprotein. The primary antibody was a monoclonal antibody against type A influenza virus nucleoprotein (kindly supplied by Dr. D.E. Swayne, USDA, ARS, Athens, GA, USA). Briefly, an antigen retrieval step was performed by pressure cooking for 25 min in citrate buffer pH 6, the primary antibody was applied at 1:2000 dilution, using the En Vision AP (DAKO K1396) detection system and Nuclear Fast Red (DAKO K1396) as chromogen.

Routine bacteriology was performed on the viscera of the affected birds.

RESULTS

Highly Pathogenic Avian Influenza

Virological investigations.

Virus isolation attempts yielded haemagglutinating agents on first passage, often accompanied by early embryo mortality (within 48 hours). Viruses were characterised serologically and all influenza isolates were characterised as type A influenza viruses of the H7N1 subtype.

The IVPI test performed on a number of isolates scored 3.0, and deduced amino acid sequence of the cleavage site of the haemagglutinin molecule was …PEIPKGSRVRR*GLF……., which contains multiple basic amino acids, a feature typical of highly pathogenic viruses.

Clinical signs.

In chickens, turkeys and guinea fowl reared on litter, 100% mortality was observed 48-72 hours from the onset of the first clinical signs. Anorexia and depression were followed by nervous signs characterised by tremors and incoordination. Similar clinical signs were recorded in pheasants although mortality rates appeared to be lower. In a limited number of broiler breeder flocks, cyanosis of the comb and wattles and petechial haemorrhages on the hock could be seen.

A different situation was noted in caged birds, such as layers and quail, in which the disease moved within the flock in a much slower way. At first, severe depression or mortality could be seen in only one bird per cage in a restricted area of the house, but slowly spread to neighbouring cages. This different behaviour in spread between caged and litter-reared birds was probably related to the amount of infected faeces in direct contact with the birds.

In free ranging ostriches (Capua *et al.,* 2000b), the first clinical signs observed were anorexia and depression in a limited number of the young birds (7-9 months), that, in the following days spread to a significant number of young birds. Common clinical findings, apart from depression and anorexia, were a swollen appearance of the throat and neck associated to nervous signs such as incoordination, paralysis of the wings and tremors of the head and neck. A consistent clinical sign was the production of brilliant green urine, which was also rich in urates and of haemorrhagic faeces. Following the onset of clinical signs, a total of 44 (30%) birds died. The remaining birds recovered (to normality) within a week from the onset of the clinical condition. The adults (breeders) appeared healthy throughout the episode.

Quite unexpectedly, mortality also affected Muscovy ducks and geese in a backyard flock (Capua & Mutinelli, 2001). The ducks had exhibited an abnormal gait associated with incoordination prior to death.

Post mortem findings.

On post mortem, a lesion which was common to all affected birds was pancreatitis. The gross finding was most severe in chickens and turkeys. Besides this finding, in chickens, occasionally, the spleen presented necrotic foci on its surface, and the caecal tonsils appeared haemorrhagic. Generally speaking, internal organs appeared congested,

and in a limited number of cases, affecting both turkeys and chickens urate deposits in the kidney could be seen.

On the contrary, the gross findings seen in the ostriches resembled infection by *Clostridium spp.* In fact, apart from oedema of the head and upper part of the neck, and presence in the oral cavity and oesophagus of bile-green mucous liquid, the most striking lesions were observed in the intestine and in the liver. Most of the intestine was affected by a severe haemorrhagic enteritis, and its lumen contained a haemorrhagic exudate and blood clots. The liver appeared enlarged with rounded margins and its surface exhibited whitish and dark brown irregular areas. As previously mentioned, in a number of birds the pancreas appeared haemorrhagic, enlarged and hardened. The kidneys also appeared enlarged, friable and contained urate deposits. The spleen also was increased in size. The lung and trachea appeared congested and the epicardium exhibited petechial haemorrhages.

With reference to the affected waterfowl, on post mortem examination both geese exhibited pancreatic lesions. In particular, in one of them the pancreas appeared enlarged, hardened and yellowish in colour. Its surface exhibited a foamy appearance with small rounded greyish vesicles. The duodenum appeared congested, and on opening it, it contained haemorrhagic material. The spleen appeared reduced in size and an inflammation of the proventriculus was also present. The heart appeared congested and enlarged. No other lesions were detected in other organs. No gross lesions were detected in the two Muscovy ducks.

Bacteriology.

Routine bacteriological tests constantly yielded a negative result.

Histopathology and immunohistochemistry.

The histopathological findings recorded in chickens and turkeys were very similar. Pancreatitis with severe, focal to diffuse necrosis of acinar cells was the main finding. Pancreatic lobes exhibited strong irregular eosinophilic staining caused by acinar necrosis and the most severe necrotic foci were lined by a thin rim of inflammatory cell debris. Interstitial oedema was also present, associated to fibrinous peritonitis affecting both pancreas and intestine. Spleen lesions were of vascular wall fibrinoid necrosis. Brain and cerebellum showed focal necrosis in affected turkeys. Lymphocytic choroiditis was also observed. No other relevant lesion was detected in other districts. Avian influenza virus nucleoprotein was identified in necrotic acinar epithelium of the pancreas, nervous and heart tissues.

In the dead ostriches, focal to diffuse coagulative necrosis of spleen, kidney and liver were detected. Inflammation and fibrinoid necrosis of the arterioles was prominent in the spleen and brain. The pancreas was affected in a limited number of birds and exhibited focal coagulative necrosis of acinar cells, restricted mononuclear infiltration and mild to severe fibrosis surrounding a few small rounded lobules which appeared compressed and atrophic. Brain and cerebellum sections revealed foci of malacia and neuronophagia. Lymphocytic choroiditis was also present. Necrotic and haemorrhagic lesions were present in the intestine. No other relevant alterations were detected

histologically. Type A avian influenza virus nucleoprotein was detected by immunohistochemistry in necrotic lesions.

Interestingly, different lesions were observed in the ducks and geese. In contrast to diffuse necrosis of affected organs observed in other birds, the main histological lesion was inflammation of the affected organs in these birds. Only a limited number of necrotic foci of the acinar cells of pancreas were observed in the geese and, to an even lesser extent in the Muscovy ducks. Mild haemorrhagic duodenitis was observed in both geese and in the Muscovy ducks, while necrosis of the cecal tonsils was observed in the geese only. The latter also presented with congestion, mild hydropic degeneration and focal granuloma in the liver. Mild to moderate lymphocytic encephalitis with perivascular cuffing was observed in the brain of geese and Muscovy ducks.

Mild positive immunohistochemical reaction against the viral nucleoprotein antigen was detected in the acinar cells of pancreas of the geese. Similarly, nuclei and cytoplasm of the neurons and astrocytes in the grey matter of the central nervous system of geese showed an intense, positive immunohistochemical reaction, while in the Muscovy ducks, a positive reaction was restricted to a few individual neurons and glial cells. Lymphocytic perivascular cuffing never showed a positive reaction on immunohistochemistry. The remaining organs for both species were negative by immunohistochemistry.

Newcastle disease

Virological investigations

Following the suspicion of ND, samples collected from all the intensive and dealer flocks, and from a significant number of backyard farms, processed for attempted virus isolation yielded a haemagglutinating agent on first passage. All samples were tested for bacterial contamination by routine methods, with negative results. Haemagglutinating agents were identified as Newcastle disease virus (CEC, 1992b), and according to the monoclonal antibody binding pattern, they were classified as viruses belonging to the C1 group (Alexander *et al.,*1997b).

In ICPI tests, a value in the range 1.6-2.0 was obtained for each of the isolates tested, confirming their virulence for chickens.

The deduced amino acid sequence of the region coding for the cleavage site of F protein …SGGRRQRR*FIG…, demonstrating the presence of multiple basic amino acids which is a characteristic associated directly with virulence (Collins *et al.*, 1993).

Clinical signs

Clinical signs in chickens were initially characterised by anorexia and depression, listlessness and ruffled feathers. In most cases nervous signs such as incoordination, tremors, opistothonus, torticollis, paralysis of the wings and nervous tics were the predominant clinical signs. Other clinical signs included severe conjunctivitis, respiratory distress, such as gasping and enteric signs dominated by the production of a brilliant green diarrhoea. Mortality rates were generally high, in some farms

approaching 100% of the birds present, with death occurring within 24-48 hours after the onset of clinical signs. In meat-type guineafowl, ND evolved as a peracute disease . Initial depression was followed by a dramatic rise in mortality, determining death of 12,000 birds (100%) in 5 days. Only few birds exhibited clinical signs with a dark green diarrhoea, nasal discharge, and death preceded by pedalling movement in a recumbent position. In turkeys clinical signs affected a limited number of birds (approximately 10-15 %) and were predominantly nervous. In pheasants, nervous and enteric signs were observed with high mortality rates in unvaccinated birds.

Only young ostriches (<1month of age), and not adults were affected by a clinical condition characterised by depression and anorexia and haemorrhagic enteritis.

Post mortem findings

In chickens, post-mortem findings were typical of ND. The most striking lesions were haemorrhages on the proventriculus and necrosis of the cecal tonsils and of the lymphnodules along the intestine. The spleen appeared enlarged and covered with pin-point necrotic foci. The lung appeared congested and the trachea was lined by a catarrhal exudate, and its mucosa exhibited petechial haemorrhages.

In guinea-fowl, only a few birds exhibited post mortem lesions, which appeared to be less evident than in chickens. Catarrhal tracheitis, with petechial haemorrhages on the trachea and pharynx, was occasionally present. Pin-point haemorrhages on the proventriculus and haemorrhagic duodenitis were seen in a limited number of cases.

Since only backyard and semi-intensive farms containing turkeys and one small ostrich farm were affected, these were not submitted for pathological investigations due to the prompt stamping out of the infected farms.

Pheasants exhibited post mortem findings similar to chickens with haemorrhages on the proventriculus, on the intestinal mucosa and to a lesser extent on the respiratory tract.

Histopathology

In chickens, individual to confluent foci of necrosis of lymphocytes were detected in the spleen and cecal tonsils. Extensive necrosis of the follicular lymphoid cells in the bursa of Fabricius was also present. Necrotic foci surrounded by lymphohistiocytic infiltrate were observed in the myocardium. Intestinal lymph nodules were affected by necrotic processes which extended to the mucosa resulting in difteroid enteritis. Haemorrhagic lesions were detected in the mucosa of the proventriculus. Catarrhal exudate occasionally covered an oedematous, haemorrhagic tracheal mucosa. Despite the nervous symptoms seen in the field, no histological lesions could be detected in the central nervous system.

Bacteriological investigations

Routine bacteriology performed on selected organs constantly gave negative results in all birds examined except for the ostriches, in which *Clostridium perfringens* was isolated.

DISCUSSION

Clinical and pathological data collected during the HPAI epidemic, confirm that gallinaceous birds are highly susceptible to HPAI, and that all efforts should be made to control this disease which may have devastating consequences on the poultry industry and on the social community.

Furthermore it appears that ostriches are also susceptible to HPAI, although it should be stated that only the young birds exhibited clinical signs, while adults remained clinically healthy. This evidence should stimulate further research aiming to establish whether adult ostriches could behave as carriers of this infection when imported for breeding purposes into countries that are free from HPAI.

Of particular interest are the findings observed in the infected waterfowl. In fact, although waterfowl are considered refractory to HPAI, from the evidence collected, it appears that in some instances they may exhibit clinical signs and experience viraemia that results in viral replication in vital organs. It should be stated that isolation of HPAI from the brain of experimentally infected Pekin ducks has been reported in the past (Wood *et al.,* 1995).

In addition to this, from the gross, histopathological and immunohistochemical data collected in the epidemic, it appears that the pancreas has a crucial role in the pathogenesis of HPAI. Further studies are necessary to elucidate the role of this organ in the pathogenesis of HPAI.

The clinical, gross and histopathological data collected during the ND epidemic indicate that chickens and guinea-fowl are highly susceptible to ND, while turkeys, pheasants and ostriches show a minor susceptibility. A major susceptibility of chickens compared to turkeys has been reported by several authors, and recently by Alexander *et al.* (1998b).

With reference to the origin of infection, this remains to be established. Italy has been experiencing an epidemic of Newcastle disease caused by the "pigeon strain" of avian paramyxovirus 1 (PPMV1), and a similar situation is occurring in other European countries (Alexander *et al.,* 1998a; Alexander *et al*. 1999b). In fact, the outbreaks reported at the European Union Reference Laboratories Meetings have been of 26 ND outbreaks in the European Union in 1998, mainly caused by group P strains (Sander, 1998; Alexander *et al.,* 1998a). Only 6 outbreaks have been reported in 1999 and no C1 group isolates were submitted in that year (Pittman, 1999; Alexander *et al*., 1999b).

In recent years , only viruses belonging to the "P" group have been isolated in Italy between 1996 and 1999, with the exception of one virulent virus, belonging to the C1 group isolated from an amateurial - hobby flock, located on the Slovenian border in 1998 (Selli & Cancellotti, 1997; Capua & Cancellotti, 1999a).

ND viruses belonging to the C1 group have caused outbreaks in Scandinavian countries (Jørgensen *et al.,* 1999; Alexander *et al*., 1997a) and in the UK in 1997 (Alexander *et al*., 1999a). Only sporadic outbreaks and no other epidemics have been reported since (Alexander *et al*., 1999b).

Although further investigations are necessary to establish whether this is the same virus, specific surveillance and monitoring should be performed in European countries, in order to detect the presence of NDV that is definitely circulating undiagnosed.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Virology and Histopathology Departments of the Istituto Zooprofilattico Sperimentale delle Venezie and Ruth Manvell, CVL Weybridge for their precious technical assistance. Jill Banks, CVL Weybridge, UK, and Ernesto Tisato Biotechnology Laboratory Istituto Zooprofilattico Sperimentale delle Venezie are gratefully acknowledged for sequencing the isolates.

The authors also wish to thank Dr. D. E. Swayne for kindly supplying the monoclonal antibody.

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SURVEILLANCE FOR PARAMYXOVIRUS TYPE 1 AND AVIAN INFLUENZA VIRUS (H5&H7) IN WILD MIGRATORY BIRDS IN SWEDEN (PRELIMINARY STUDY).

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Introduction

Following outbreaks of Newcastle disease in 1995 and 1997 in Sweden, and an outbreak of highly pathogenic avian influenza (HPAI) in Northern Italy during the winter of 1999/2000 a study was initiated to evaluate the presence of paramyxovirus type 1 (PMV-1) and avian influenza viruses (H5&H7) (AIV) in the wild avifauna in Sweden.

Materials and Methods

The study was performed during the spring of 2000. In total, 150 serum samples and 120 cloacal swabs were obtained from 53 migrating bird species (orders *Anseriformes, Charadriiformes, Columbiformes, Strigiformes, Piciformes* and *Passerinformes*) (Table 1) at Ottenby Bird Station on southern Öland (an island in the Baltic off the south-east coast of Sweden). The swabs were obtained from the same, but not all, individual birds as the serum samples. The bird species were selected based on their winter habitat, in order to sample migratory birds which were likely to have either spent the previous winter in Italy or visited Italy while migrating northwards. Additionally, cloacal swabs from Anseriformes in southern Sweden will be analysed during the spring of 2001.

The serum samples were tested by hemagglutination-inhibition test for the presence of antibodies to AIV, and by a blocking ELISA (SVANOVIR® Svanova Biotech, Uppsala, Sweden) for antibodies to PMV-1. All cloacal swabs were cultured according to Council directive 92/66/EEC and 92/40/EEC. Following each of the three passages the allantoic fluid was tested for hemagglutinating (HA) virus.

Results

One serum sample from a Ringed Plover (*Charadrius hiaticula*) showed a low titer (1:16) against AIV (H5). None of the serum samples were positive for PMV-1, however three Ringed Plovers showed PI-values of 40%. No cloacal swabs were available from these four birds. No viruses were isolated from any of the cloacal swabs.

Discussion

A similar surveillance has been carried out in 1994. Five hundred cloacal swabs were collected from different migrating bird species. A lentogenic PMV-1 (ICPI 0.0, mab serogroup L) was isolated from a black-headed gull (*Larus ridibundus*).

The negative result of the present study indicates a low prevalence of PMV-1 and AIV in the wild migratory avian population during early spring but may also reflect to the low number of samples tested.

However, even if very few birds are infected with AIV or PMV-1 on arrival in Scandinavia, the infection may spread within bird populations during the breeding season. In order to identify the true prevalence of these viruses in the Scandinavian wild avifauna, the number of samples, the selection of species as well as the optimal time for sampling should be re-evaluated.

Table 1. List of sampled bird species

Latin name	English name	Number of samples
Order *Anseriformes*		
Anas platyrynchos	Mallard	3
Anas querquedula	Garganey	2
Branta canadensis	Canada Goose	2
Clangula hyemalis	Long-tailed Duck	1
Somateria mollissima	Common Eider	10
Order *Charadriiformes*		
Actitis hypoleucos	Common Sandpiper	9
Arenaria interpres	Ruddy Turnstone	6
Caladris alba	Sanderling	1
Calidris alpina	Dunlin	17
Calidris ferruginea	Curlew Sandpiper	7
Calidris minuta	Little Stint	2
Calidris temminckii	Temminck's Stint	2
Charadrius dubius	Little Ringed Plover	2
Charadrius hiaticula	Ringed Plover	6
Larus argentatus	Herring Gull	5
Larus canus	Common Gull	1
Larus marinus	Great Black-backed Gull	10
Larus ridibundus	Black-headed Gull	1
Limicola falcinellus	Broad-billed Sandpiper	10
Limosa limosa	Black-tailed Godwit	3
Philomachus pugnax	Ruff	2
Tringa glareola	Wood Sandpiper	6
Tringa nebularia	Greenshank	1
Tringa totanus	Redshank	13
Order *Columbiformes*		
Columba palumbus	Common Wood Pigeon	1

Latin name	English name	Number of samples
Order *Strigiformes*		
Asio otus	Long-eared Owl	1
Order *Piciformes*		
Dendrocopos major	Great Spotted Woodpecker	1
Picus viridis	Green Woodpecker	1
Order *Passeriformes*		
Anthus trivialis	Tree Pipit	2
Carduelis carduelis	Goldfinch	7
Carduelis chloris	Greenfinch	2
Carduelis flammea	Redpoll	2
Carduelis spinus	Siskin	1
Coccothraustes coccothraustes	Hawfinch	5
Corvus monedula	Jackdaw	2
Delichon urbica	House Martin	1
Erithacus rubecula	Robin	11
Ficedula hypoleuca	Pied Flycatcher	1
Fringilla coelebs	Chaffinch	5
Fringilla montifringilla	Brambling	1
Lanius collurio	Red-backed schrike	2
Luscinia svecica	Bluethroat	2
Motacilla alba	White Wagtail	5
Phoenicurus phoenicurus	Redstart	13
Phylloscopus trochilus	Willow Warbler	4
Prunella modularis	Hedge Accentor (Dunnock)	1
Sylvia atricapilla	Blackcap	3
Sylvia communis	Whitethroat	2
Sylvia curruca	Lesser Whitethroat	2
Turdus iliacus	Redwing	1
Turdus merula	Blackbird	5
Turdus philomelos	Song Thrush	12
Turdus pilaris	Fieldfare	2

HIGHLY PATHOGENIC AVIAN INFLUENZA (H7N1) AND NEWCASTLE DISEASE IN ITALY

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Avian influenza H7N1 epidemic

During 1999, North eastern Italy was affected by an epidemic of low pathogenicity avian influenza (LPAI) caused by a virus of the H7N1 subtype. The epidemic involved 199 farms and caused considerable losses to the poultry industry. In the month of December 1999, the H7N1 LPAI virus mutated to a highly pathogenic avian influenza virus (HPAI), which spread rapidly, affecting 413 farms, and resulting in direct or indirect death of over fourteen million birds of different species.
The data concerning the HPAI epidemic that occurred in Italy during 1999 and 2000 are presented in Tables 1 and 2.

Other avian influenza isolates
An avian influenza isolate, obtained from imported passerine birds was received from the Istituto Zooprofilattico della Lombardia e dell'Emilia, Brescia, Italy. The isolate was typed as H3N8.

Table 1: Total number of outbreaks and species affected during the 1999-2000 H7N1 HPAI epidemic in Italy

	Turkey breeders	Turkey meat type	Guinea fowl	Quail, Ducks, Pheasants	Ostrich	Broilers	Layers	Broiler breeders	Backyard flocks
No. Outbreaks	**5**	**177**	**9**	**5**	**3**	**39**	**121**	**29**	**25**
No. Birds	**42 276**	**2 692 917**	**247 379**	**260 340**	**387**	**1 625 628**	**8 118 929**	**743 319**	**1 737**

Table 2: Highly pathogenic avian influenza in Italy (17.12.1999 – 05.05.2000) – species and category of birds

Region	Broilers	Layers	Broiler breeders	Backyard flocks	Turkey Breeders	Turkey meat type	Guinea fowl	Quail, Ducks, Pheasants	Ostrich	**Total**
Veneto	14	21	8	6	2	102	2	1	2	158^
Lombardia	25	97	21	4	3	72	7	4	1	234§
Friuli Venezia Giulia	-	1	-	1	-	3	-	-	-	5
Piemonte	-	1	-	5	-	-	-	-	-	6
Trentino	-	-	-	1	-	-	-	-	-	1
Sicilia	-	-	-	2	-	-	-	-	-	2
Sardegna	-	1	-	-	-	-	-	-	-	1
Emilia Romagna	-	-	-	5	-	-	-	-	-	5
Umbria	-	-	-	1	-	-	-	-	-	1
Total Outbreaks	**39**	**121**	**29**	**25**	**5**	**177**	**9**	**5**	**3**	**413**
Total Animals	**1,625,628**	**8,118,929**	**743,319**	**1,737**	**42,276**	**2,692,917**	**247,379**	**260,340**	**387**	**13,732,912**

^ The total includes 8 pre-emptively slaughtered flocks that resulted virologically positive
§ The total includes 21 pre-emptively slaughtered flocks which resulted virologically

Newcastle Disease

Italy has been affected by an epidemic of Newcastle disease, caused by velogenic virus characterised as C1, which caused a total of 254 outbreaks. The details on the number of outbreaks and birds involved per region are reported in Table 3.
A total of 29 pigeon paramyxovirus isolates have been obtained during 2000. Of these 23 were isolated from free ranging pigeons and 6 from collared doves *(Streptopelia decaocto).* All isolates were identified by means of mAb 161-617, and exhibited ICPI values of ranging from 0.5 to 1.4.

Table 3. Newcastle disease outbreaks in Italy April-December 2000 – species an category of birds.

Region	Broilers	Guinea fowl	Layers	Backyard flocks	Ostriches	Meat-type turkeys	Dealers	**Total**
Emilia Romagna	2	1	2	31			4	40
Friuli V.Giulia	1			20	1			22
Lazio				7				7
Lombardia				19		1*	2	22
Marche	1			14			4	19
Piemonte	5	1	1					7
Toscana	1			109			7	117
Trentino				4				4
Umbria				13				13
Veneto	1			2				3
Total Outbreaks	11	2	3	219	1	1	17	254
Total Animals	**400,917**	**34,270**	**177,663**	**12,227**	**65**	**2,500**	**106,039**	**773,681**

* Virus isolation at the slaughterhouse

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AVIAN INFLUENZA AND NEWCASTLE DISEASE EPIDEMICS IN ITALY DURING 1999 AND 2000: A REVIEW

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INTRODUCTION

Avian Influenza (AI) and Newcastle disease (ND) are two viral diseases of poultry included in OIE List A. In the European Union their control is imposed by EU Directives 92/40/EEC and 92/66/EEC, respectively (CEC, 1992a; CEC, 1992b). These two diseases may have devastating effects on the poultry industry particularly following the high mortality rates they determine in susceptible birds, but also their presence in a given territory results in restrictions on animal movements, marketing and trade of poultry and poultry products.

North-eastern Italy has been affected by a devastating epidemic of highly pathogenic avian influenza (HPAI), caused by a type A influenza virus of the H7N1 subtype that originated from the mutation of a low pathogenicity avian influenza (LPAI) virus of the same subtype (Capua & Marangon, 2000). The LPAI epidemic and the subsequent HPAI epidemic occurred in the Veneto and Lombardia regions, which raise 65% of Italy's industrial poultry. Furthermore, some areas affected by the epidemics (particularly south of Verona province), are densely populated livestock areas (DPLA), which count (in some municipalities of Verona province) 70 000 birds raised per square kilometre.

The HPAI epidemic determined directly or indirectly the death or culling of over 14 million birds that inevitably determined the disruption of the marketing system and great economic losses to the poultry industry and to the social community. The dramatic economic and social problems determined by the epidemic indirectly led to the introduction of Newcastle disease, that for obvious reasons determined to additional losses and trade restrictions.

Following depopulation and restocking of the HPAI infected areas, LPAI re-emerged, thus determining the poultry industry to request and obtain vaccination against avian influenza of the H7 subtype. The events which connect the four epidemics mentioned above are, in our opinion linked together, and are reported below.

Low pathogenicity avian influenza epidemic. On the 29th of March 1999 the first isolation of a type A, H7 avian influenza virus was officially notified. The virus was further characterised, in accordance to EU Directive 92/40/EEC (CEC 1992a), by the EU Reference Laboratory for Avian Influenza and Newcastle Disease, Weybridge U.K., as a type A avian influenza virus of the H7N1 subtype. The intravenous pathogenicity index (IVPI) of the isolate, in 6 week-old SPF chickens was of 0.0, and the deduced amino acid sequence of the genome segment which encodes for the cleavage site of the precursor of the haemagglutinin molecule was typical of LPAI viruses since it did not contain multiple basic amino acids (Wood *et al.*, 1994; Wood *et al*., 1997).

Following the first official notification a significant number of outbreaks were diagnosed and notified for a total of 199 infected flocks. The highest number of outbreaks affected meat turkeys (164), and only a limited number of turkey breeder flocks were affected (6). Infection also affected chickens (12 outbreaks in layers, 11 in broiler breeders and 4 in broilers) and two guinea-fowl flocks. From the epidemiological inquiry it appeared that at the moment of the first submission approximately 60-70 turkey farms had already been infected. Infection was particularly severe in the turkey industry, causing severe losses to farmers (Capua *et al.,* 1999).

Nevertheless, this virus did not have the characteristics listed in EU Directive 92/40/EEC, therefore no compulsory stamping out policy could be implemented, and it was not possible at the time to stamp out such a consistent number of flocks on a voluntary basis. Moreover, since LPAI is not considered in Italian veterinary legislation, there were no legislative tools to prevent its spread. However, the regional authorities of the two affected regions, implemented restriction orders with the aim of reducing the number of new outbreaks. The main strategies of these orders were to avoid movement of viraemic birds, and to avoid movement of dead birds and infected litter, which were identified as being among the primary sources of infection. These policies, aided by the oncoming warm season, determined a decrease in the number of outbreaks during the summer, that inevitably increased from the month of September.

Highly pathogenic avian influenza epidemic. On the 13th of December, 1999 a private practitioner submitted pathological samples from a meat turkey flock exhibiting high mortality rates. The outbreak was confirmed as HPAI on the 17th of December with the characterization of an H7N1 isolate with an IVPI index of 3.0 and a deduced amino acid sequence containing multiple basic amino acids, typical of highly pathogenic viruses (Capua *et al.,* 2000).

Due to the complex field situation (isolation of an H7 virus was not unusual at the time) it was not possible to suspect immediately the presence of HPAI virus when it first appeared and to promptly implement eradication measures, thus resulting in spread of infection. Furthermore, the holiday season was approaching and high slaughter levels resulted in a further spread of the virus with complete loss of control of infection. 413 outbreaks were diagnosed involving 177 meat turkey flocks, 121 table-egg layer flocks, 39 broiler flocks, 29 broiler breeder flocks, 25 backyard flocks, 9 guineafowl flocks, 6 turkey breeder flocks, 3 ostrich farms, 2 pheasant flocks, one Pekin duck flock and one quail flock and death of over 14 000 000 birds. The last outbreak was notified on April 5th, 2000.

As a result of mass mortality, (stamping out policy and pre-emptive slaughter), several establishments such as hatcheries, feed mills, abattoirs, processing plants and other connected activities were forced to interrupt their activity, causing unemployment and heavy economic losses to the poultry industry and to the social community, due to the disruption of the marketing system. Further economic losses were also determined by the export bans imposed on the infected regions and by the depopulation of the infected area.

Eradication of HPAI. Following the implementation of Directive 92/40/EEC (CEC, 1992a) infected flocks were stamped out, and cleaning and disinfection of infected premises was carried out. To improve eradication procedures, a complete depopulation of the infected area was imposed. An area of 5500 square kilometres was depopulated, including intensive, semi-intensive and backyard flocks, which remained empty for a minimum period of 60 days. Restocking began on June 15th 2000.

Newcastle disease epidemic. Due to mass mortality, and to unavailability of chicks on the Italian market, day-old chicks and hatching eggs were imported from several European and non-European countries. These imported batches originated from different countries with different sanitary statuses and were mingled in the same hatcheries. Veterinary controls were reduced on imports due to the urgent and rising demand.

Furthermore, in AI free areas, stocking densities were increased, resulting in poor environmental conditions for the birds. In addition, due to the high stocking densities, and to the fact that the immune status to ND of the imported chicks was generally unknown, vaccination programs for ND were reduced or abandoned.

The first industrial flocks that were affected by ND all originated from the same broiler hatchery. Infection spread to other industrial flocks, to dealers (who sell all sorts of poultry to backyard farm owners), and subsequently to backyard flocks, for a total of 17 outbreaks in industrial farms, 17 in dealers, 219 in backyard flocks and one ostrich flock.

The ND strains involved had an intracerebral pathogenicity index ranging between 1.6 and 2.0 and a deduced aminoacid sequence at the cleavage site of the F protein of …SGGRRQRR*FIG…, which contains multiple basic amino acids, a feature typical of virulent viruses. It was subsequently typed with a panel of 28 monoclonal antibodies as a virus belonging to the C1 group (Alexander *et al.,*1997).

Following the implementation of Directive 92/66/EEC, all infected flocks were stamped out, and at present we only have occasional outbreaks in backyard poultry.

Re-emergence of LPAI. On the 14th of August, 2000 a clinical suspicion of LPAI was forwarded in a turkey flock located in the DPLA, and was confirmed by the laboratory on August 20th, 2000. The Italian Ministry of Health ordered the eradication of infection with a stamping out policy imposed by an extraordinary act. Fifty-two outbreaks were diagnosed and stamped out.

A vaccination policy against avian influenza was, at this point, strongly requested by the farmers and by the poultry industry, and a vaccination program was drawn up and approved by the European Commission.

Vaccination policy. The vaccination program began on November 15th 2000 and will last until May 2002. 6,000,000 birds [only meat type birds and table-egg layers (that apply the all-in all-out system)] raised in a restricted zone (1155 km^2) south of Verona have been

vaccinated. No vaccinated live birds or poultry products that originate from the vaccination zones will be authorised for intra-community trade.

The vaccine that has been used does not contain a homologous H7N1 virus, but has been prepared from an inactivated H7N3 virus (A/CK/Pakistan/95/H7N3). The reason for this is the possibility of using it as a natural "marker" vaccine, or more correctly a DIVA [Differentiating Infected from Vaccinated Animals] vaccine. In fact, the presence in the vaccine of an H7 antigen ensures protection against clinical signs and the reduction of virus shedding, since it is well known the neutralising antibodies to influenza A viruses are induced primarily by the haemagglutinin molecule (Swayne *et al.,*1999). The presence of a different neuraminidase (N) subtype, which will induce specific antibodies (against N3 rather than N1) will enable us, with the aid of an *"ad hoc"* diagnostic kit, to discriminate between infected and vaccinated flocks, and to monitor and follow the evolution of the situation.

DISCUSSION

A few considerations can be made from this experience. Firstly, farmers and private companies should bear well in mind that within the current European legislation there is no financial aid from local or national governments or from the European Union in case of LPAI. Therefore, on one hand permanent surveillance programs should be implemented in order to allow the prompt diagnosis of infection by H5 and H7 LPAI viruses, to allow the stamping out of infected flocks until this is economically feasible. In the spring of 1999 we were faced with 60-70 outbreaks and it was not possible to stamp out infected flocks without compensation.

The spread of infection was also a result of the structure and organisation of the local poultry industry. In several areas worldwide, the poultry industry has substantially grown in an often irrational way, particularly where the system has developed as a semi vertical integration. The latter, (i.e. house owned by the farmer and day-old chicks and feed supplied by private company) has the disadvantage that there is no planning behind the spatial distribution of the units that are involved in the system, and furthermore, there are a sensible number of contacts between establishments. In fact, frequently feed trucks and other vehicles (e.g. abattoir delivery), visit a number of farms daily, regardless of the species reared and of the type of production, and basic biosecurity measures are rarely respected. The concentration of poultry houses, hatcheries, abattoirs, litter processing plants and other establishments in a restricted area is definitely convenient from an organisational point of view, but has a series of drawbacks from the sanitary point of view, that dramatically emerge when an epidemic of a highly contagious disease is faced.

The disruption of the marketing system, determined social consequences, forcing farmers out of business and in some instances favouring the use of illegal vaccines. This practice most probably determined the re-emergence of LPAI, through the movement of infected litter collected from farms containing clinically healthy carriers.

Furthermore, the commercial pressure posed on the companies, determined imports at risk, which associated to insufficient veterinary controls, managing inaccuracies and weak vaccination programs led to the emergence of ND.

With reference to AI the vaccination program, this is being used as a last resort, although there are conflicting opinions on its effectiveness as an eradication tool.

In conclusion, the Italian experience with avian influenza shows that it is extremely difficult to control avian influenza in densely populated areas, especially if infection with LPAI is already widespread in the area, and therefore in order to avoid similar situations prevention systems should be implemented.

With reference to ND , the analysis of epidemiological data, highlighted two relevant factors. Firstly, that hatcheries may play an important role in the spread of Newcastle disease. Although vertical transmission of ND has only been reported in a limited number of instances (Pospisil *et al*., 1991; Capua *et al.,* 1993), this possibility may not be ruled out. However, since it is well known that NDV reaches high concentrations in faeces, egg-shell contamination with infected faeces could represent the means of entry into the hatchery.

In addition, the role of dealers or "svezzatori" in disseminating and perpetuating infection has been identified, the role of this category of retailers has also played an important role in the HPAI epidemic which occurred in north eastern Italy during 1997 and 1998, caused by a type A virus of the H5N2 subtype (Capua *et al.,*1999), and may be considered similar to the role live bird markets have in the USA (Trock, 1997).

In our opinion a further point has emerged from this epidemic. The restrictions imposed by EU Directive 92/66/EEC (CEC, 1992b) include a protection zone of 3 km of radius and a surveillance zone of 10 km of radius regardless the size of the flock affected. In the epidemic, which occurred in Italy, the greatest number of outbreaks was notified in backyard flocks and a significant number of protection and surveillance zones were drawn, with a consistent amount of energy and effort by the public veterinary services. Furthermore, these zones often caused movement restrictions on the intensive farms, which were located inside them. In our experience, outbreaks in backyard flocks are self-limiting, and moreover, after the stamping out of the infected flock has occurred, it is very unlikely that infection may spread further. It could therefore be suggested that different restriction measures could be applied to backyard flocks, leaving in force the ones listed in Directive 92/66/EEC for dealers and intensive reared poultry. The application of less stringent measures for backyard flocks would have the advantage on one hand to reduce the workload on public veterinarians and the other to avoid economic losses to farmers who intensively rear poultry in the restriction zones.

Besides a structural change in the industrial system which must inevitably take place in order to reorganise production circuits, veterinary surveillance, quarantine and controlled marketing particularly in restocking procedures are also essential to prevent sanitary emergencies. In addition to this, education of farmers and staff to the basic concepts of biosecurity is also a critical point to the eradication of avian influenza and Newcastle disease and are fundamental to the management of intensively reared poultry.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Virology and Histopathology and Epidemiology Departments of the Istituto Zooprofilattico Sperimentale delle Venezie and of the Istituto Zooprofilattico della Lombardia e dell'Emilia Romagna.

D.J.Alexander, R.J.Manvell and J.Banks of the EU Reference Laboratory for Avian Influenza and Newcastle Disease, Weybridge U.K. are also gratefully acknowledged for their support and technical assistance.

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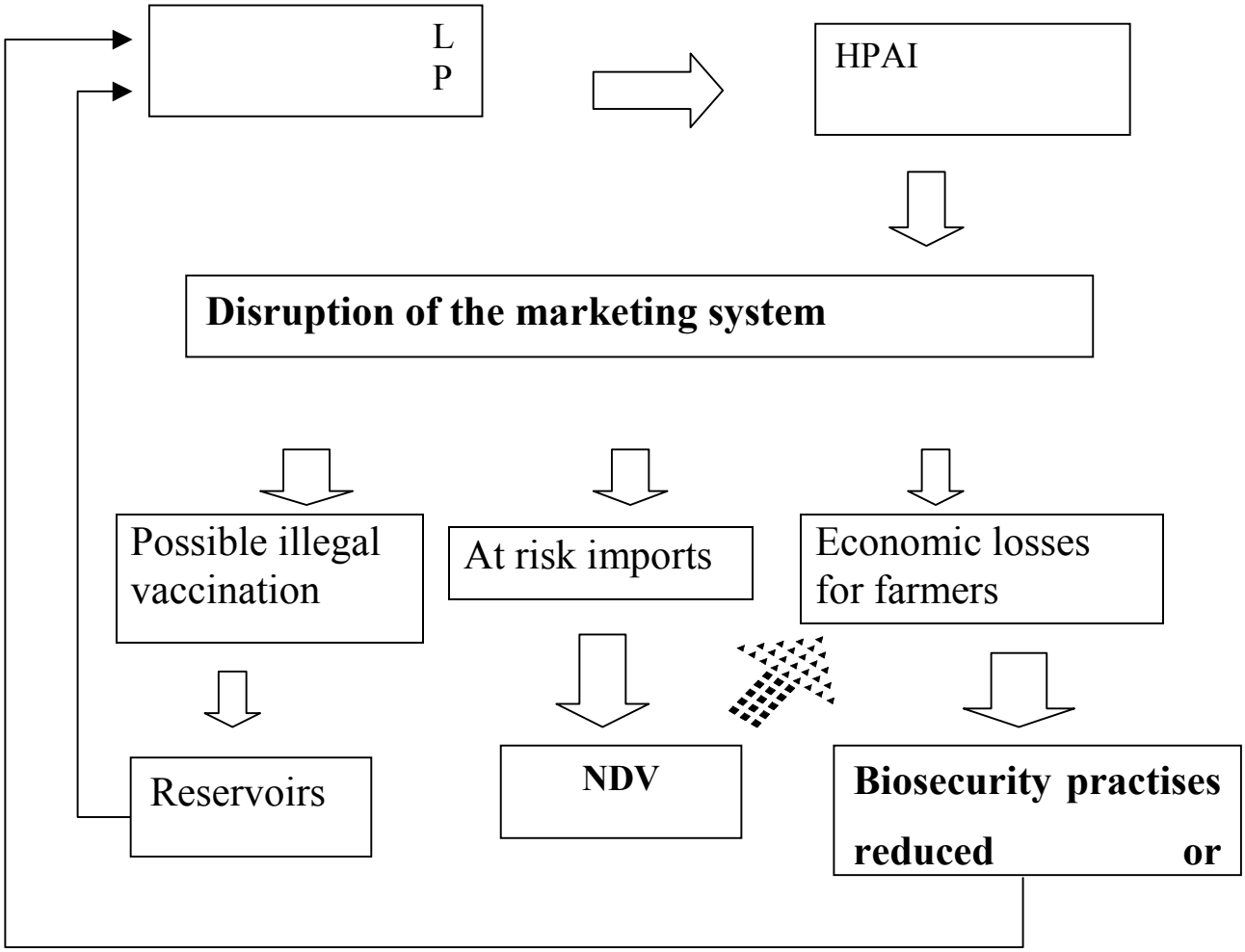

Fig. 1 Connections between the LPAI and HPAI and NDV epidemics in Italy, 1999-2000

SURVEILLANCE FOR AVIAN INFLUENZA IN GERMANY

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There is no official surveillance programme for avian influenza in wild birds or in poultry supported by the German government or by the European Union.
Since the avian influenza outbreaks in Northern Italy in 1999/2000 accompanied with high economic losses, the awareness of the poultry producers and the poultry veterinarians for avian influenza has increased. Therefore in many areas of Germany with high poultry density voluntary investigations were established. In addition more samples for investigation for avian influenza were submitted to the regional laboratories and their diagnostic activity has increased.
The National Reference Laboratory distributed inactivated H7 antigen for Haemagglutination Inhibition tests among all regional Laboratories and gave detailed instructions for laboratory diagnosis of avian influenza.
Here are presented the results of serological monitoring of meat turkey and of laying hen flocks and also the results of serological and virological investigations of all regional laboratories in Germany in 2000.

The Federal Poultry Producer Association in Germany has decided to monitor all slaughtered turkey flocks and in Lower Saxony also all laying hens and some broiler flocks for influenza A antibodies. At the time of slaughter 9 – 10 blood samples per flock are collected and examined for avian influenza antibodies using commercial ELISA test kits (IDEXX) or Immuno Diffusion Test.
Samples that have reacted positively in these group specific tests are submitted to the National Reference Laboratory and examined in Haemagglutination Inhibition test (HI) using subtype specific antigens (Figure 1).

In the year 2000 a total number of 19 959 samples from about 2000 turkey flocks were examined (Table 1). 155 samples were positive, this equals 0.8 %. Most of them came from Lower Saxony. Out of these 155 positive samples 110 revealed antibodies to H6. These 110 samples represent 15 different flocks. 45 samples derived from 6 different flocks revealed antibodies to H1. Antibodies to H7 or H5 were never detected.
The 15 523 chicken sera samples came from approximately 1600 layer flocks and 25 broiler flocks taken at slaughter. Only 9 samples, all from the same layer flock, were positive and had antibodies to H1. All other sera had no antibodies to Avian Influenza virus.

Additionally to the monitoring financed by the Federal Poultry Producer Association the following sera samples were examined in the HI test for antibodies to Subtype H7 in the regional laboratories (Table 2). The chicken and turkey sera mostly came from smaller flocks and from animals of different age and using type. Also sera from ducks, geese and ostriches were collected in small flocks. For examination a maximum of 5 samples per flock were submitted, but in some cases single samples only. The samples of herring

gulls were yolk samples of eggs, collected in a brooding colony near the Insel Riems. They were tested in the National Reference Laboratory.

All investigated sera and yolk samples were negative for Avian Influenza virus antibodies subtype H7.

In table 3 the summarised results of attempts for isolation of Avian Influenza virus and Newcastle Disease virus in Germany in the year 2000 are shown.
There was no specific suspicion for avian influenza or Newcastle disease in any case, but dead animals were submitted for the investigation to find out the cause of death. Therefore in all cases of poultry and of pet birds organ samples were processed and inoculated in embryonated eggs and/or in cell cultures.
The investigated capercaillies or wood grouses were imported birds died during quarantine. Most other wild birds were found dead in the wild and submitted to the veterinary laboratories by the finder. In many cases accidents were the cause of death. 20 crows were shot in different geographic regions of Saxony within the scope of a small surveillance programme.
The samples of small wild birds consist of 5 dead birds and 198 samples of faeces collected at bird feeding tables.

No Avian Influenza virus was isolated, neither from the 2 391 poultry samples nor from the pet birds nor from the 337 wild birds.

The results indicate that avian influenza virus H7 and H5 subtypes are not circulating in poultry flocks in Germany. However in some areas other influenza subtypes seem to be present.

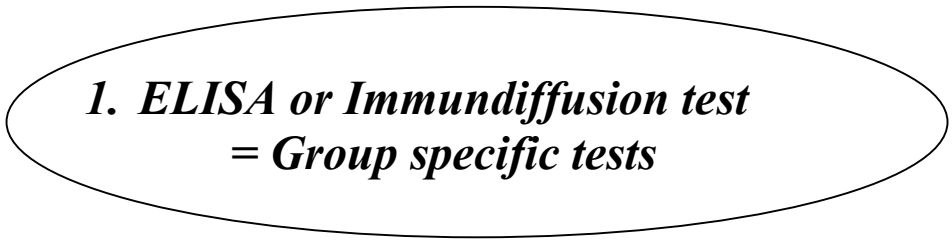
This has been the situation in Germany until the early 2001.

In March 2001 in a small mixed flock of free ranging poultry near Munich one out of two turkeys died. A haemagglutinating virus was isolated from the organ samples, which was detected not to be PMV 1.
In the National Reference laboratory the virus was characterised as avian influenza virus of subtype H7N7, unlike the Italian virus with the antigens H7N1. The pathogenicity of the virus was low. The intravenous pathogenicity test revealed an index of 0.03 and the cleavage site of the haemagglutinin showed an amino acid sequence typical for low pathogenic virus. After collection of sera samples the small flock of 20 animals was stamped out and the entire equipment was disinfected or burnt. Eight out of the 20 samples were positive for H7 antibodies. All poultry flocks, which had direct or indirect contact, were serologically monitored. One small flock of 18 hens was found to be positive. It was stamped out too. The serological monitoring is still going on. The source of the H7N7 virus is unknown. No new birds were added to the flock during the last months. The keeper reported that wild ducks from the nearby ponds came often to the poultry to eat. Therefore it is assumed that wild ducks are probably the source of the introduction of the virus.

This special case emphasises the necessity of surveillance of wild birds especially of wild water birds.

Figure 1

Serological Examination



allows the detection of antibody to all AIV Subtyps

If samples were positive

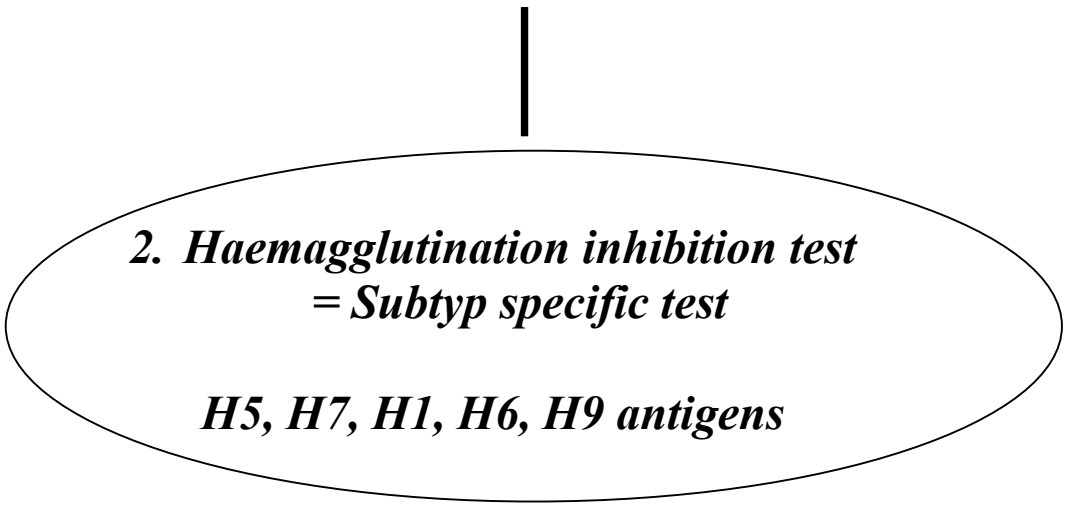


Table 1

Results of Monitoring of Turkeys and Chickens for AIV

	Turkeys	**Chickens**
EIA		
Samples tested	***17,773***	***7,555***
Positive	***155***	***9***
Subtype	***110 x H6*** ***45 x H1***	***9 x H1***
IDT		
Samples tested	2,186	7,968
Positive	0	0
Total tested	19,959	15,523

Table 2

Sporadic Serological Investigation for AIV Subtyp H7 in Germany in 2000

Bird species	**Number of Samples**
Chicken	***1030***
Turkey	***2145***
Duck	***73***
Goose	***227***
Ostrich	***40***
Pigeon	***18***
sea gull	***27***

Results: All sera were negative

Table 3

Results of Trials on Isolation of AIV or NDV in Germany in 2000

Species	Number of birds	Number of Isolates	
		AIV	PMV 1
Chickens	*1204*	*0*	*30*
Turkeys	*67*	*0*	*0*
Ducks	*82*	*0*	*0*
Geese	*50*	*0*	*1*
Ostriches	*8*	*0*	*0*
Ornamental poultry	*59*	*0*	*1*
Pigeons	*598*	*0*	*84*
Parakeets/parrots	*262*	*0*	*8*
Orn. birds (small)	*61*	*0*	*3*
Capercaillies (Import)	*27*	*0*	*7*
Wild birds (small)	*203*	*0*	*0*
Crows/ravens	*23*	*0*	*2*
Birds of prey / owls	*67*	*0*	*0*
Cranes	*6*	*0*	*0*
Storks	*8*	*0*	*0*
Great bustards	*2*	*0*	*0*
Swan	*1*	*0*	*0*
total	***2728***		

TOPICS FOR DISCUSSION

THE DEFINITION OF NEWCASTLE DISEASE

PROBLEM

The Belgium National Laboratory [representatives of which could not be present at the meeting] asked for the disparity between the OIE definition of Newcastle disease [ND] and that currently in use in the EU to be brought to the attention of the Meeting and discussed.

The current definition of ND is that of Council Directive 92/66/EEC [CEC 1992]. This states that the definition of ND for which control measures should be imposed when birds are affected in European Union [EU] countries is:
"an infection of poultry caused by an avian strain of the paramyxovirus 1 with an intracerebral pathogenicity index [ICPI] in day-old chicks greater than 0.7"

However, the current OIE definition adopted in May 1999 [OIE 2001] is:

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

a. *The virus has an intracerebral pathogenicity index in day-old chicks (Gallus gallus) of 0.7 or greater. OR*
b. *Multiple basic amino acids have been demonstrated in the virus (either directly or by deduction) at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term 'multiple basic amino acids' refers to at least three arginine or lysine residues between residue 113 and 116. Failure to demonstrate the characteristic pattern of amino acids as described above would require characterisation of the isolated virus by an ICPI test.*

The problem caused by this difference in definition related to PPMV-1 isolates obtained from pigeons giving low ICPI values, sometimes less than 0.7 despite having cleavage site sequences ^{113}RQKRF^{117} characteristic of virulent viruses. The problem has been particularly noticeable in Belgium where viruses with ICPI values less than 1.0 isolated from pigeons in 1998 and 1999 were the rule rather than an unusual observation [Meulemans et al 1999; 2000]. The Belgium laboratory pointed out that these viruses fell within the OIE definition but not the EU definition of ND and when notified to OIE some trading countries were applying restrictions.

DISCUSSION

Attention was drawn to the "Definition of ND" report adopted by the EU Scientific Committee on Animal Health and Animal Welfare [SCAHAW, 1998] on 24.03.98 The Committee recommended the definition of Newcastle disease should be as follows:

"Newcastle Disease" is defined as an infection of poultry caused by a virus of avian paramyxovirus serotype 1 (APMV-1) which has an intracerebral pathogenicity index (ICPI) in day-old chicks (Gallus gallus) of 0.7 or greater.

As an alternative to the ICPI test, the presence of "Newcastle Disease" virus can also be confirmed by the demonstration (either directly or by deduction) of multiple basic amino acids [at least three arginine or lysine residues between residues 113 to 116] at the C-terminus of the F2 protein and phenylalanine [F] at residue 117 which is the N-terminus of the F1 protein. Failure to demonstrate the presence of multiple basic amino acids or F at 117 would require characterisation of the isolated virus in an ICPI test.*

While this addressed the discrepancy between the OIE and EU reports relating to viruses with ICPI values lower than 0.7 but with multiple basic amino acids at the F0 cleavage site, it was further pointed out that existing and putative EU definitions referred to infections of "poultry" and the OIE definition "birds". The SCAHAW report also suggested "poultry" should be redefined as "*all birds which are reared or kept in captivity for breeding, the production of meat or eggs for consumption, the production of other commercial products or for restocking supplies of game*". The meeting was uncertain whether or not this included racing pigeons. It was also mentioned that the OIE had intended to alter the Animal Health Code to compartmentalise different types of birds, but that this had not yet been done.

RECOMMENDATIONS

The Meeting urged the Commission to adopt the definition of ND put forward by the SCAHAW and to clarify the classification of APMV-1 infections of pigeons.

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WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2002

Working Document SANCO/1655/2001 Rev. 1

I. LEGAL FUNCTIONS AND DUTIES

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

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2002

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

   a) Determining the intravenous pathogenicity index (IVPI)

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

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

   d) Limited phylogenetic analysis to assist in epidemiological investigations.

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

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

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

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

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

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

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

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

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

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

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2002

I. LEGAL FUNCTIONS AND DUTIES

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

II. OBJECTIVES FOR THE PERIOD JANUARY – DECEMBER 2002

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

    a) Determining the intracerebral pathogenicity index (ICPI)

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

    c) Antigenic grouping of viruses

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

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

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

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

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

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

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

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

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

ANNEX I

COMMUNITY FINANCIAL ASSISTANCE PROVIDED TO CRLs IN ANIMAL HEALTH AND ZOOTECHNIC 1998 – 2001

CRLs	BUDGET 1998*	BUDGET 1999**	BUDGET 2000***	BUDGET 2001
Avian Influenza	40.000	30.000	70.000	75.000
Newcastle Disease	94.000	75.000	55.000	55.000
Classical Swine Fever	150.000	180.000	185.000	46.000(b)
Swine Vesicular Disease	55.000	80.000	94.000	95.000
African Horse Sickness	20.000	——	40.000	40.000
Fish Diseases	94.000	120.000	120.000	125.000
Diseases of bivalve molluscs	83.000	90.000	90.000	90.000
Rabies serology	——	——	40.000(a)	130.000
Bluetongue	——	——	——	115.000
Assessment of bovine breeding	20.000	40.000	50.000	60.000
TOTAL	**556.000**	**615.000**	**744.000**	**831.000**

(a) From July - December 2000

(b) From October - December 2001

* Com. Decision 98/587/EC OJ L 282, 20.10.1998, p. 73.

** Com. Decision 1999/587/EC OJ L 223, 24.8.1999, p. 28.

*** Com. Decision 2000/293/EC OJ L 95, 15.4.2000, p. 40.

COMMISSION DOCUMENTS

CONTROL OF AVIAN INFLUENZA IN THE EU AND IMPLICATIONS ON INTERNATIONAL TRADE

Working Document SANCO/2436/2001

INTRODUCTION

Avian Influenza (AI) is a viral disease of poultry and wild birds. The disease is classified as a "List A" disease by the International Animal Health Code of the Office International des Epizooties (OIE). This classification means that the disease:

– has the potential for very serious and rapid spread, irrespective of national borders;

– is of serious socio-economic importance;

– is of major importance in the international trade of live poultry, poultry meat, eggs and other products originating from poultry.

In the Manual of Standards for Diagnostic Tests and Vaccines published by OIE the criteria for classifying avian influenza viruses as highly pathogenic are the following:

a) Any influenza virus that is lethal for six, seven or eight of eight 4-8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid.

b) The following additional test is required if the isolate kills from one to five chickens but is not of the H5 or H7 subtype: growth of the virus in cell culture with cytopathogenic effect or plaque formation in the absence of trypsin. If no growth is observed, the isolate is considered not to be a HPAI isolate.

c) For all H5 and H7 viruses of low pathogenicity and for other viruses, if growth is observed in cell culture without trypsin, the amino acid sequence of the connecting peptide of the haemagglutinin must be determined. If the sequence is similar to that observed for other HPAI isolates, the isolate being tested will be considered to be highly pathogenic.

For the purpose of the diagnostic procedures for the confirmation and differential diagnosis of avian influenza within the European Union the following definition was adopted by Directive 92/40/EEC;
"Avian influenza" means an infection of poultry caused by any influenza A virus which has an intravenous pathogenicity index in six-week-old chickens greater than 1.2 or any infection with influenza A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the cleavage site of the haemagglutinin.
The purpose of this paper is briefly to review the measures applied by the EU for the control and eradication of Avian Influenza, and to provide information on the disease situation and to describe trade conditions.

1. CONTROL AND ERADICATION OF AI

a) Control measures

The measures adopted to control and eradicate AI 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 the virus. To ensure such actions in the event of an outbreak, Member States have within the framework of Council Directive 92/40/EEC the 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 include requirements for:

- designation of national laboratories and a Community reference laboratory;
- 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 that is not compatible with the Single Market.

To facilitate a decision to regionalise 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;

– the boundary of the region must be easily controlled;

– 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. The level of compensation is normally up to 50% of Member States' costs, which relate to the 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 2000 adopted several Decisions concerning Community financial assistance related to the control of AI.

2. DISEASE SITUATION

Avian Influenza is reported by Member States in accordance with the provisions of Council Directive 82/894/EEC. Since the adoption in 1992 of Council Decision 92/40/EEC the Member States of the EU have reported the occurrence of Avian Influenza on three occasions. In 1992 a single outbreak (H5 N1) was recorded in the UK; in 1997/98 Italy reported 8 outbreaks (H5 N2) and in 1999/2000 Italy experienced an epidemic (>413 outbreaks) caused by the H7 N1. The presence in Italy of a LPAI H7 N1 virus in poultry prior to the emergence of the HPAI virus appears to be a significant factor during the evolution of the epidemic.

3. INTERNATIONAL TRADE

Until 1995 those concerned about poultry diseases in relation to trade adopted frequently a zero risk policy. If there was a perception of threat, no matter how little or unsubstantiated, the door to trade (importation) was firmly closed. In this context it should be mentioned that the International Animal Health Code of the OIE considers a country/zone to be free or infected in accordance with the conditions listed blow:

a) HPAI free country

A country may be considered free from HPAI when it has been shown that HPAI has not been present for at least the past 3 years.

This period shall be 6 months after the slaughter of the last affected animal for countries in which a stamping-out policy is practised with or without vaccination against HPAI.

b) HPAI infected zone

A zone shall be considered as infected with HPAI until:

i) at least 21 days have elapsed after the confirmation of the last case and the completion of a stamping-out policy and disinfection procedures, or

ii) 6 months have elapsed after the clinical recovery or death of the last affected animal if a stamping-out policy was not practised.

With regard to trade conditions for Avian Influenza the OIE code indicates that Veterinary Administrations of importing countries should require similar arrangements to those provided in the Code for Newcastle Disease. In the code (article 2.1.15.4), the following text is published:

Veterinary Administrations of ND free countries may prohibit importation or transit through their territory, from countries considered infected with ND, of the following commodities:

i) domestic and wild birds;

ii) day-old birds;

iii) hatching eggs;

iv) semen of domestic and wild birds;

v) fresh meat of domestic and wild birds;

vi) meat products of domestic and wild birds which have not been processed to ensure the destruction of the ND virus;

vii) products of animal origin (from birds) intended for use in animal feeding or for agricultural or industrial use.

With the establishment in 1995 of the World Trade Organisation (WTO) the rather imprecise approach to international trade was replaced by a more rational system based on epidemiology and level of real risk as outlined in the Sanitary and Phytosanitary (SPS) Agreement of the WTO.

The SPS Agreement defines the rights, obligations and responsibilities of Member Countries concerning trade and human, animal and plant health. It is clearly designed to facilitate trade while minimising the threat of imported disease. Its articles define how Member Countries can take measures for their own protection, as long as they are scientifically justified. These measures should be harmonised, as far as possible, with those of other countries and be based on international standards and guidelines developed by OIE. Higher standards can be used if they are scientifically reasonable. Members should accept another Member's measures, even if they differ, as long as they achieve the same level of disease protection. Sanitary measures should be based on risk assessments, which conform to internationally recognised methods. The measures should take into account regional conditions and recognise disease free areas or areas of low risk within countries. On the other hand Members, claiming such areas, must provide the necessary evidence. Members should notify the international community, OIE, of changes in their animal disease status and provide information on their sanitary measures. Any control measures, inspections, and approvals must be done without undue delay, and be no less favourable for imported than for domestic products. The SPS agreement deals also with administration, implementation, dispute settlement, and special provisions for countries of the developing world. The key elements in this system are efficient disease monitoring and surveillance, effective disease control, open information exchange, and measures taken on a proper scientific basis.

4. EU ANIMAL HEALTH CONDITIONS FOR TRADE

The general animal health requirements applicable to intra-Community trade in and imports from third countries of poultry and hatching eggs are laid down in Council Directive 90/539/EEC whilst the requirements for similar trade in fresh poultry meat are laid down in Council Directive 91/494/EEC. The provisions of Directive 90/539/EEC relate to requirements concerning:

– the approval of hatcheries and of establishments for breeding and rearing of poultry;

– surveillance for certain specific poultry diseases;

– the animal health status of hatching eggs, day-old chicks and poultry prior to dispatch;

– vaccines, and

– animal health certificates.

In case of trade in fresh meat the requirements listed in Directive 91/494/EEC refers in particular to:

– the animal health status of the flocks of origin;

– transport of poultry to the slaughterhouse;

– the health mark given to fresh poultry meat, and

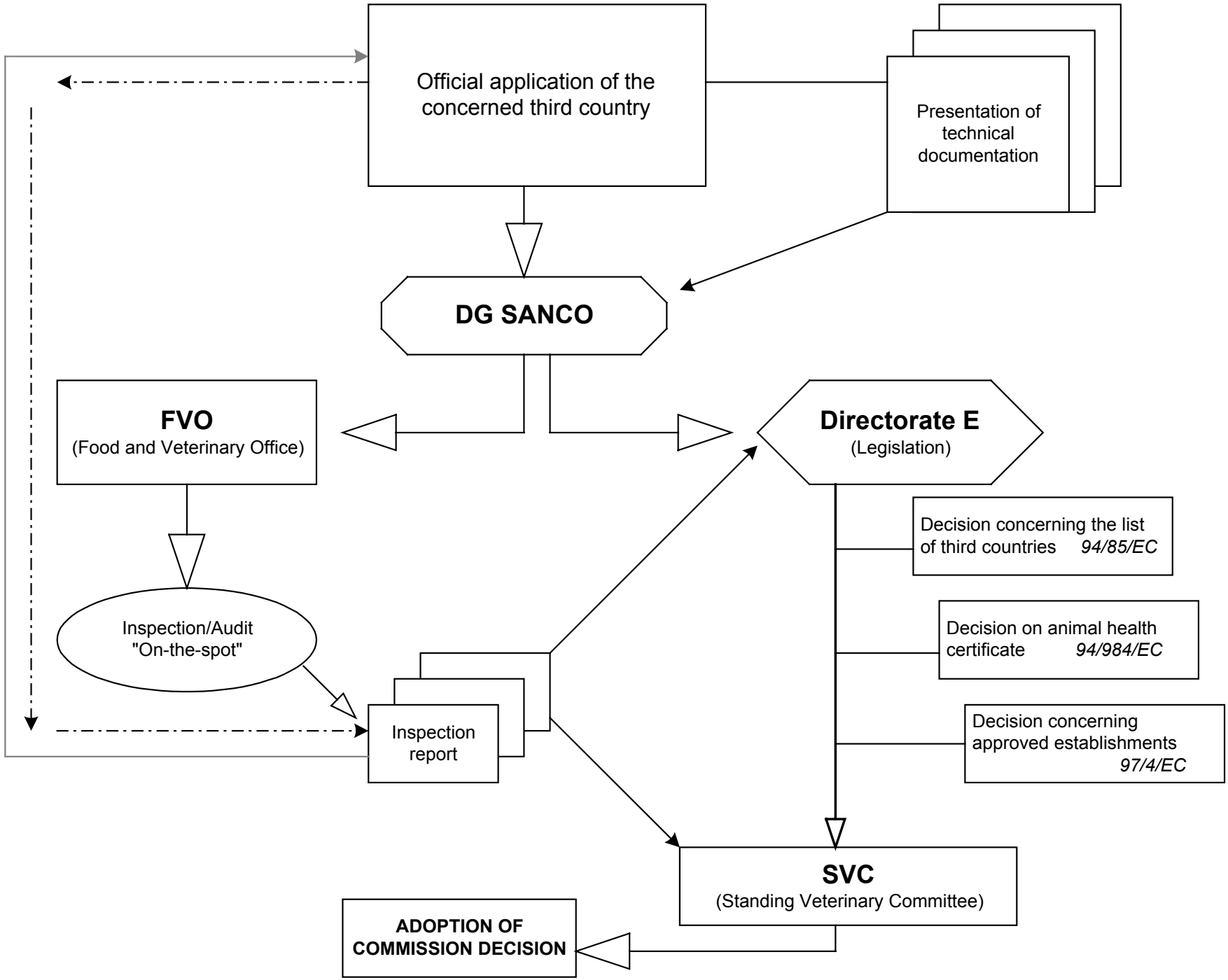
– animal health certificates.

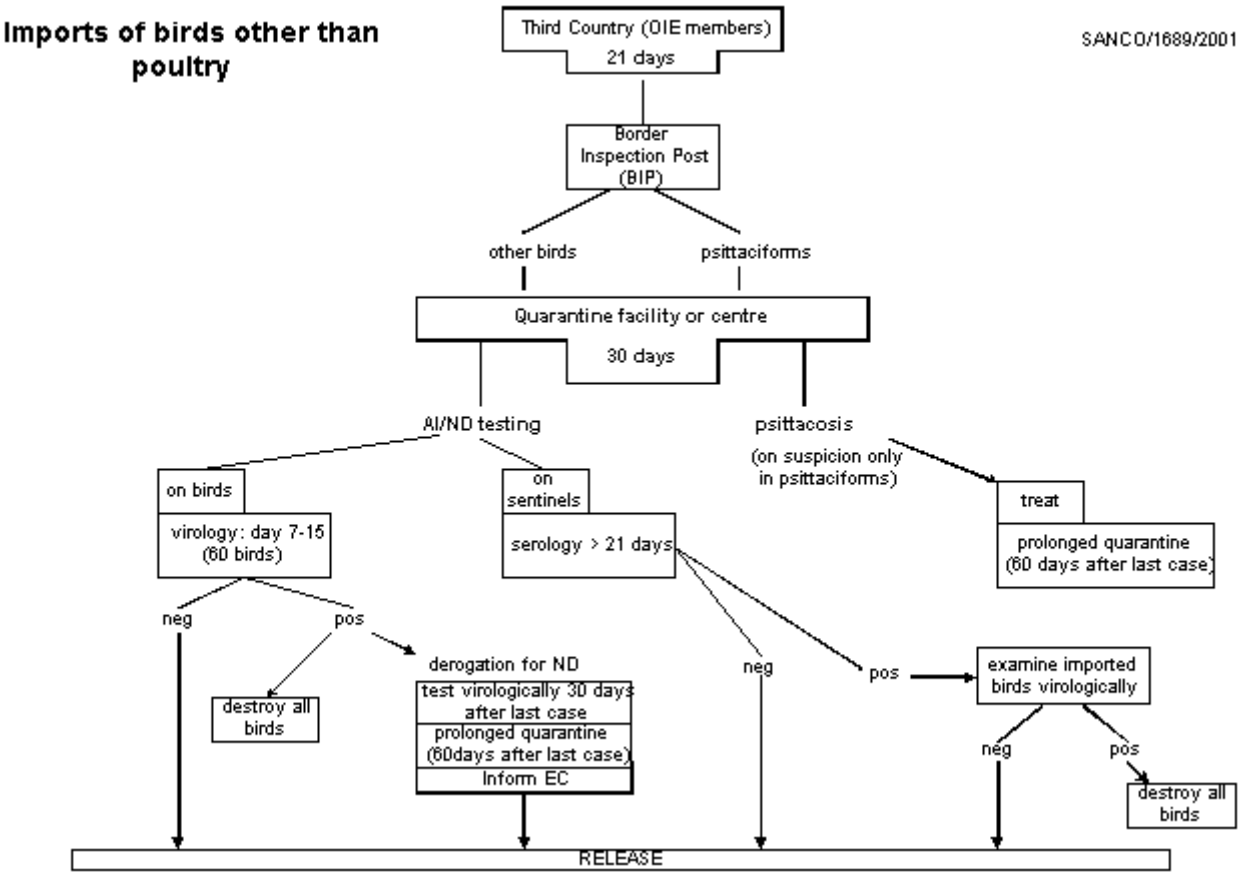
The animal health requirements with regard to protection against spread of avian influenza virus are well defined in the two trade directives. With regard to intra-Community trade hatching eggs, day-old chicks, live poultry and fresh poultry meat must not be subject to restrictions due to suspicion or occurrence of Avian Influenza. With regard to imports from third countries the commodities must come from a country free from Avian Influenza or which, although not free from Avian Influenza, applies measures to control the disease which are at least equivalent to those laid down in Council Directive 92/40/EEC.

The practical arrangements to be carried out in relation to import of fresh poultry meat from third countries are illustrated in Figure 1.

Procedure for the authorisation of imports of fresh poultry meat

Commission Document – Control of AI in the EU





The trade in ornamental birds for the pet animal market originating from countries all over the world also pose a risk for the introduction of poultry diseases into the territory of the Community, therefore the sanitary requirements for such imports were harmonised by Commission Decision 2000/666/EC laying down the animal health requirements and the veterinary certification for the import of birds, other than poultry and the conditions for quarantine. Figure 2 shall demonstrate the procedure, which is to follow in case of such imports.

5. IMPLICATIONS ON TRADE CAUSED BY AVIAN INFLUENZA

In Italy during 1999/2000 the outbreaks of Avian Influenza had severe implications on trade due to movement restrictions on live poultry, fresh poultry meat and hatching eggs in areas defined by provisions in EU legislation and in national legislation.

The EU legislation was in particular the following:

– Council Directive 92/40/EEC introducing Community measures for the control of Avian Influenza;

– Commission Decision 2000/149/EC concerning certain protection measures relating to Avian Influenza in Italy;

– Commission Decision 2000/721/EC on introducing vaccination to supplement the measures to control Avian Influenza in Italy and on specific movement control measures.

The economic losses to the Italian Community due to outbreaks of Avian Influenza in 1999/2000 are difficult to calculate. Within the framework of Council Decision 90/424/EEC compensation can be made for animals killed and destroyed, destruction of contaminated feed, cleaning and disinfection of holdings and contaminated equipment. The tentative estimates for such losses is about 140 M EURO whilst other costs (indirect costs) related to movement restrictions have been estimated to about 230 M EURO. In addition to the costs directly affecting the Italian Community some Member States have experienced difficulties in maintaining the export to certain third countries.

6. FUTURE DEVELOPMENTS

In the light of the experiences gained during the Italian epidemic of avian influenza from December 1999 until May 2000 the Scientific Committee on animal health and animal welfare was asked for its opinion, if the definition for avian influenza adopted by Council Directive 92/40/EEC is still appropriate or if it should be modified. Furthermore the Committee was requested to review the possible benefits of using vaccination for the control of the disease.

The full content of the report is available on the following web site of the internet:

http://europa.eu.int/comm/food/fs/sc/scah/outcome_en.html

a) Definition of Avian Influenza

Taking into account that HPAI viruses can emerge from LPAI viruses of H5 and H7 subtypes by mutation the Scientific Committee on animal health and animal welfare recommended that the current definition should be amended in such way to include all infections of avian influenza subtypes H 5 and H 7 irrespective their further characterisation in the definition which calls for eradication measures.

b) Use of vaccination

The Scientific Committee stated in its report that the current legislation as regards vaccination should be maintained. This means that vaccination against avian influenza H 5 and H 7 subtypes should only be carried out in case of an outbreak of avian influenza as an emergency vaccination and not as a prophylactic measure. It is assumed that vaccination could mask an ongoing infection whereas the clinical symptoms of infection are suppressed.

Nevertheless the development of marker vaccines to distinguish between infected and vaccinated animals should be enhanced.

c) Surveillance

Throughout the European Union there is a lack of surveillance for avian influenza in poultry and particularly in free living birds. Routine surveillance in free living birds could give an early warning of the prevalence of viruses of H5 and H7 subtype in the locality of domestic birds. There is also a need for a point prevalence assessment of the current situation of infection of poultry with viruses of H5 and H7 subtypes.

The Scientific Committee therefore gave the recommendations that Member States should put in place routine surveillance systems for the detection of influenza viruses in free living birds and should undertake serological surveys of poultry, also in order to assess the economic impact of a change of the definition for avian influenza as described under a).

d) High density

With regard to the future of the poultry industry it is evident that the industry should reassess the advantages and disadvantages of producing poultry in areas with a high concentration of animals. It has for some time been recognised that in areas of high livestock density there are multitudes of potential risk factors hampering the rapid eradication of viral diseases. Unrecognised virus replication in flocks with direct or indirect contact with infected flocks may lead to further spread of virus and new outbreaks within and beyond restricted areas.

e) Contingency plans

To avoid major epidemics in the future it is important that contingency plans are well rehearsed, an effective disease awareness is present, movement records are available and that there is a complete participation and dedication of all those engaged in the poultry industry to prevent the spread and to control infectious diseases.

INFORMATION ON SURVEILLANCE FOR AVIAN INFLUENZA IN WILD BIRDS CARRIED OUT IN MEMBER STATES

Working Document SANCO/3829/2000 Rev.2

DETECTION OF INFLUENZA VIRUS IN FREE LIVING BIRDS DURING 1999 AND 2000

Examination carried out and results obtained from avian influenza surveillance in free-living birds

Member State	Species	Number of birds	Specimens examined	Number of birds virus positive	Virus detected
Austria	No survey carried out				
Belgium	No survey carried out				
Denmark	No survey carried out				
Finland	Duck	2	organs	0	
	Swan	1	and/or	0	
	Gull	4	intestinal	0	
	Canadian goose	1	contents	0	
	Oystercatcher	1		0	
	Pigeon	3		0	
	Eider	2		0	
	Auk	2		0	
Finland total	8	16		0	
France	No data received				
Germany	Pigeon	120	Organs	0	
	Songbird	23	-"-	0	
	Pheasant	2	-"-	0	
	Quail	4	-"-	0	
	Wild Duck	4	-"-	0	
	Crow	5	-"-	0	
	Swan	1	-"-	0	
	Birds of Prey	42	Swabs	0	
	Owl	4	-"-	0	
	Capercaillie	1	-"-	0	
	Gull	27	Egg yolk	0	
Germany total	11	233		0	
Greece	Seagull	8	Faeces, Brain, Trachea, Lungs, Spleen	0	
	Finches	12		0	
	Sparrows	10		0	
Greece total	3	30		0	

Member State	Species	Number of birds	Specimens examined	Number of birds virus positive	Virus detected
Ireland	No survey carried out				
Italy	Owls	2	Organs	0	
	Brambling	10		0	
	Dove	**9**		**1**	**H7N1**
	Goose	1		0	
	Flamingo	3		0	
	Pigeon	5		0	
	Crow	2		0	
	Heron	5		0	
	Swan	1		0	
	Sparrow	**8**		**2**	**H7N1**
	Thrush	1		0	
	Lapwing	3		0	
	Cormorant	1		0	
	Wild ducks	25		0	
	Other Species	27		0	
Italy total	>15	103		3	
Luxembourg	No survey carried out				
Netherlands	Mainly Ducks and Geese	3789	Cloacal Swabs and faeces	1-2% in PCR	**37 viruses** H1,H2,H3,H4 **H5(non-virulent)** H6,H11,H13
NL total	2	3789			
Portugal	No data received				
Spain	Pigeon	22	Organs	0	
	Duck	10	-"-	0	
	Partridge	25	-"-	0	
	Swan	1	-"-	0	
	Sparrow	1	-"-	0	
Spain total	5	59			

Member State	Species	Number of birds	Specimens examined	Number of birds virus positive	Virus detected
Sweden	Longtailed duck	1			
	Chaffinch	5			
	Ruff	20			
	Eider	10			
	Herring gull	5			
	Wild Duck	3			
	Greenfinch	5			
	Green Woodpecker	3			
	Siskin	5			
	Black-backed gull	10			
	Longeared owl	1			
	House-martin	1			
	Jackdaw	2			
	Canada geese	2	Organs	0	
	Blackbird	5			
	Ringdove	1			
	Redwing	5			
	Readbreast	11			
	Blacktailed godwit	3			
	Redstart	4			
	Goldfinch	7			
	Hawfinch	6			
	Cormorant	41			
	Wagtail	5			
	Songtrush	6			
	Tawny pipit	5			
	Garganey	2			
Sweden total	27 species	174		0	
United Kingdom	No data received				

DIRECTORY OF NATIONAL LABORATORIES

LIST OF MEMBER STATES' NATIONAL LABORATORIES FOR AVIAN INFLUENZA

According to Annex IV of Council Directive 92/40/EEC
(updated at the Annual meeting of the AI/ND Laboratories in
Uppsala, Sweden, 26-28 April 2001)

Working Document SANCO/2762/2001

ANNEX IV

LIST OF NATIONAL AVIAN INFLUENZA LABORATORIES

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Belgium & Luxembourg:
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TEL: +39 049 808 4369
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(updated at the Annual meeting of the AI/ND Laboratories in Uppsala, Sweden, 26-28 April 2001)

Working Document SANCO/2763/2001

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APOLOGY

The editor would like to apologise for the delay in producing these proceedings. This was due to an unanticipated increased workload during 2001.