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Health and Consumer Protection
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PROCEEDINGS OF THE JOINT SIXTH ANNUAL MEETINGS OF THE NATIONAL NEWCASTLE DISEASE AND AVIAN INFLUENZA LABORATORIES OF COUNTRIES OF THE EUROPEAN UNION

HELD IN BRUSSELS, 29th-30th NOVEMBER 1999

Edited by Dennis J. Alexander

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SIXTH JOINT ANNUAL MEETINGS OF EUROPEAN UNION NATIONAL NEWCASTLE DISEASE AND AVIAN INFLUENZA LABORATORIES 1999

PROGRAMME

Monday 29th November 1999

10.00: Meet at Room 00/S38 86, Rue de la Loi, Brussels.

10.30: Introduction and aims of meeting

COUNTRY REPORTS FOR 1999 - AVIAN INFLUENZA

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

Current situation of avian influenza in invited non-EU countries [Norway, Poland, Hungary. Slovak Republic, Slovenia, Czech Republic, Estonia, Latvia, Bulgaria, Lithuania, Cyprus, Romania, Switzerland].

General discussion of current avian influenza situation in Europe and the rest of the World

Lunch will be between 12.30 and 14.00

COUNTRY REPORTS - FOR 1999 - NEWCASTLE DISEASE

1. Greece
2. Spain
3. Portugal

4. Italy
5. France
6. Belgium/Luxembourg
7. The Netherlands
8. Germany
9. Sweden
10. Finland
11. Denmark
12. Ireland
13. United Kingdom
14. Austria

Current situation of Newcastle disease in invited non-EU countries: Norway, Poland, Hungary, Slovak Republic, Slovenia, Czech Republic, Estonia, Latvia, Bulgaria, Lithuania, Cyprus, Romania, Switzerland.

General Discussion of Current Situation of Newcastle disease in Europe and the rest of the World

about 15.30: BREAK

16.00: Results of the comparative virus identification tests in different laboratories. *Dennis Alexander*

17.00: **Close**

Tuesday 30th November 1999

10.00: Meet at Room 00/S38 86, Rue de la Loi, Brussels

10.15: **ORIGINAL CONTRIBUTIONS**

10.15: Ability of NP- and M-based RT-PCR tests to detect type A avian influenza viruses *Veronique Jestin*

10.35: PCR-based screening assay (MP genes) to detect all subtypes of AI known to date, comparison of the sensitivity of this screening method with propagation in eggs. *Ron Fouchier*

11.00: The low pathogenicity avian influenza (H7N1) epidemic in the Veneto region, Italy. *Ilaria Capua*

EU REPORTS

11.45: Report of the EU Reference Laboratory for avian influenza and Newcastle disease 1999 *Dennis Alexander*

12.15: Report from the Commission on aspects of Newcastle disease and avian influenza legislation in the European Union:

- Animal health requirements related to trade in ratites and ratite meat (Doc.XXIV/2950/99) *Kirsten Sander*
- Aspects on Newcastle disease and Avian influenza legislation (Doc.XXIV/2913/99) *Maria Pittman*

12.45: LUNCH

14.00: DISCUSSION OF THE FOLLOWING TOPICS

- Crimean Congo haemorrhagic fever *Ilaria Capua*
- proposed EU and OIE new definitions of ND again
- H9N2 influenza virus infections
- emergence of virulent ND virus in Australia
- APMV-1 in pigeons
- comparative test for 2000
- other topics raised during the meeting
- The functions and duties of Reference Laboratories (Doc.XXIV/2912/99) *Jorgen M. Westergaard*
- miscellaneous

about 15.30: Recommendations, closing remarks and close.

COUNTRY REPORTS FOR 1999

AVIAN INFLUENZA

Representatives of the following countries made statements of no avian influenza outbreaks during 1999:

Spain
Ireland
Austria
United Kingdom
Finland
Denmark
Portugal
Norway
Poland
Hungary
Slovak Republic
Slovenia
Czech Republic
Estonia
Bulgaria
Romania
Switzerland

AVIAN INFLUENZA IN THE NETHERLANDS 1999

Guus Koch

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There was a single isolation of an avian influenza virus of low pathogenicity from a turkey flock in The Netherlands close to the border with Germany. The isolation was not associated with high mortality. The virus was shown to be of H1 subtype and had an intravenous pathogenicity index in six-week-old chickens of 0.0. There were numerous pig farms in the vicinity of the turkey farm, although it is not known whether there was contact or not between the pig and turkey farms.

AVIAN INFLUENZA: SITUATION IN GREECE 1999

John Papanikolaou

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No cases of avian influenza as it is defined in Directive 92/40/EEC were confirmed during 1999. Although respiratory disease signs and drop in egg production occurred in turkey and fowl breeder flocks, AI viruses were not isolated on investigation.

300 serum samples collected from different turkey and chicken flocks and tested for AI antibodies by AGP tests were negative.

The diagnostic methods for AI conform in detail to the recommendation of EU Directive 92/40/EEC.

Vaccination against AI is prohibited in Greece and no vaccine is licensed.

AVIAN INFLUENZA: THE SITUATION IN FRANCE DURING OCTOBER 1998 TO OCTOBER 1999

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No avian influenza outbreaks as it is defined in the E.U. directive 92/40/EEC were reported during the relevant period. The incidence of avian influenza virus infections of domestic poultry was extremely low. Only one isolate was obtained from a turkey breeder flock showing an egg drop problem, without increased mortality, in October 1999 and located in Finistère. The virus belonged to the subtype H6. Its IVPI was 0,00. Positive serological reactions were confirmed using HI tests.

A diagnostic laboratory has carried out serology by immunodoublediffusion test (AGP) from 27 flocks (Table 1). No consistent anamnestic data are available concerning each flock to know in which circumstance they were tested. But most of them were tested for export or egg drop. Only two flocks of turkeys had positive sera, one with 2/82 positive and the other with 10/30 positive, which is a more consistent result. Both flocks had egg drop problems.

Concerning ostriches and emus, only two sera out of 70 were positive, with such a result it can not be concluded that it is really positive or negative.

Table 1 : Avian Influenza Serology (AGP)

Species	Country (Department)	N° of flocks	Results
Chicken	Côtes d'Armor Pyrénées Atlantiques Ille et vilaine	4 1 1	Negative
Turkey	Côtes d'Armor Drôme Marne	7 2 8	1 (2+/82 sera) 1 (10+/30 sera) The rest negative
Duck	Seine et Marne	1	Negative
Guinea fowl	Côtes d'Armor	1	Negative
Ostriches emus	Loire Atlantique	1	2+/70 sera
Crane	Haut-Rhin	1 (4 sera)	Negative

AVIAN INFLUENZA - SITUATION IN GERMANY 1995 - 1999

Ortrud Werner

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Based on the definition given in Council Directive 92/40/EEC, there were no cases of avian influenza in Germany in the period under review. However, 4 isolates were made during 1998 and up to now 3 isolates during 1999 (Table 1). The pathogenicity of all of them was low.

Since the middle of 1998 there have been only H6 isolates. They came from different regions of Germany.

The subtyping of neuraminidase was performed in the EU Reference Laboratory. The different neuraminidase combinations of the H6 isolates are remarkable.

There were probably no epidemiological connections between the cases except for the two H6N2 cases.

The clinical signs in the cases caused by the viruses of subtype H6N5 and H6N1 were very mild.

In turkey flocks from which H6N2 viruses were isolated, respiratory symptoms and egg drop had been observed.

Serological surveillance of turkeys in Lower Saxony with the IDEXX-ELISA revealed antibodies to influenza virus in some flocks. Most of them could be determined as antibodies to H6 by HI test, but a few flocks had antibodies to H1. We never found antibodies to H5 and to H7.

Table 1. Isolation of avian influenza viruses from poultry in Germany 1995 - 1999

Year	Number	Virus	Subtype	IVPI
1995	3	turkey/Germany/95	H9N2	0
	1	chicken/Germany/90/95	H9N2	0
	1	duck/Germany/113/95	H9N2	0
1996	6	turkey/Germany/96	H9N2	0
1997	0	-	-	-
1998	1	chicken/Germany/45/98	H9N2	0
	3	turkey/Germany/41-43/98	H6N5	0
1999	1	turkey/Germany/4/99	H6N2	0
	1	turkey/Germany/26/99	H6N2	0
	1	turkey/Germany/30/99	H6N1	0

AVIAN INFLUENZA: COUNTRY REPORT FOR SWEDEN 1999

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Avian Influenza

During 1999 a total of 468 samples, including all imported grandparent flocks, were tested for antibodies to AI-virus, no case of avian influenza was reported. No clinical sign of Avian Influenza in any type of bird, has occurred in Sweden from January 1999 up to the present day.

ISOLATION OF A H5N2 NON-PATHOGENIC AVIAN INFLUENZA VIRUS IN BELGIUM IN 1999.

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One H5N2 isolate was obtained from a backyard flock of 100 chickens. The chickens experienced 10% mortality associated with clinical signs such as depression, diarrhoea and respiratory distress. These clinical signs appeared 10 days after introduction into the flock of 10 chickens purchased from a merchant at a local market.

The nucleotide sequence of 704 bp of the HA gene of the virus showed 94% homology with A/Duck/Postdam/1402/6-86; 93% with A/Duck/HongKong/205/77 and 92% with A/Duck/HongKong/698/79.

The amino acid sequence at the cleavage site of the HA gene was **PQKETRGLF** which is typical of non-pathogenic strains. The IVPI was 0.00.

Despite the fact that the virus was non-pathogenic, the Belgian veterinary services decided to slaughter and destroy all chickens in order to prevent further circulation of the virus and an eventual augmentation of its virulence.

A serological examination of the ducks held by the merchant was performed. All ducks were negative for H5 antibodies but 6/20 were positive for H7 antibodies. (Table 1).

Our observation confirms that chicken merchants can be a source of introduction of influenza viruses into poultry as they are keeping different species of birds together such as ducks and chickens.

Table 1. Results of serological examinations of ducks held by the merchant.

Serum	Influenza H5N2	Influenza H7N1
1	neg.	**16**
2	neg.	neg.
3	neg.	neg.
4	neg.	neg.
5	neg.	neg.
6	neg.	neg.
7	neg.	neg.
8	neg.	neg.
9	neg.	neg.
10	neg.	neg.
11	neg.	neg.
12	neg.	neg.
13	neg.	**16**
14	neg.	**16**
15	neg.	neg.
16	neg.	**16**
17	neg.	neg.
18	neg.	neg.
19	neg.	**32**
20	neg.	**16**

THE EPIDEMIOLOGICAL SITUATION OF AVIAN INFLUENZA IN ITALY DURING 1999

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Low pathogenic avian influenza viruses, subtype H7N1, responsible for 58 outbreaks of avian influenza, have been recorded in intensive farms of turkey breeders, meat turkeys, chicken breeders and broilers situated in some provinces of Lombardy (Brescia, Mantova and Cremona), Veneto (Verona) and Emilia Romagna (Ravenna) since March 1999. These regions in Northern Italy have been affected by the incidence of outbreaks in different measures (Table1), yet the marked density of poultry in the Regions concerned was a common characteristic, as 75-80% of intensive reared poultry in Italy is located in these areas. Moreover, it is important to note that, in the above provinces, cases of virus isolation were found in areas where there were large farms of different types (breeders, layers, broilers, meat turkeys, breeding and meat guinea fowls, ducks etc), incubators, slaughterhouses, collection and treatment centres of poultry manure in restricted areas. Furthermore, it is worth noting that intensive pig farming is highly developed in many of these areas.

Influenza virus type A, subtype H7N1 was consistently found to be low pathogenic in IVPI tests and the deduced amino acid sequence of the region coding for the cleavage site of the haemagglutinin molecule was typical of viruses of low pathogenicity. However, there was a different sequence in the latter characteristic in some of the strains isolated since April in Lombardy and Emilia Romagna which proved to be quite frequent. The outbreaks diagnosed through the isolation and identification of the virus affected more frequently the farms of turkey breeders and meat turkeys, while farms of chicken breeders and broilers were affected to a lesser extent. However, many clinical-serological diagnoses were carried out on, amongst others, breeding and meat guinea fowl (two outbreaks were found) but the virus was not isolated. Therefore, there is a certain numerical disparity between the records of official outbreaks (relating to outbreaks where the virus was isolated and identified) and the serological diagnoses after clinical signs. It is probable that the former are only the tip of the iceberg in a much more complex general situation.

As far as the spread of the virus is concerned, apart from a notable persistence in the areas stated above, until now there has not been any significant widening of the epizootic into other areas. Within some of the affected areas there were guinea fowl farms in the vicinity of infected turkey farms where serological monitoring (as the farms are supplied by open pens and so are considered particularly at risk) showed that the guinea fowl had not developed antibodies. Today it is generally hypothesised that the spread of the virus for the most part is due to the treatment of the infected litters, workers' shoes and vehicles. Further proof of the fact that the virus has not spread widely comes from the data concerning the light breeder chicken farms situated in the areas where there is a risk of infection and where the biosafety standards are not optimum. Indeed, they did not give seropositive results during the epidemiological monitoring programme carried out in the relative regional monitoring. It is worth noting that in one of the influenza epizootic "nests" between Lombardy and Veneto there is a collection and treatment centre for poultry manure that is used by many of the farms affected.

Some of the main breeding farms have pointed out that the only path to follow is a voluntary stamping out of the infected breeders which would have been more successful if integrated with other measures concerning the eradication of the virus. Particular attention must be paid to layers farms where the practice of "all full - all empty" is seldom applied.

The sero-epidemiological monitoring programmes carried out have revealed a different picture in each region. In particular, in Lombardy (Table 2) where 340 farms were monitored, 10.6% resulted positive, and in these 62.2% of the poultry tested had developed specific antibodies against H7. The positivity was greatest in turkey farms (57% of the farms tested), followed by heavy breeders (17% of the farms tested) and farms of layers (5.7% of the farms tested).

The sero-epidemiological monitoring programme carried out in Veneto is summarized in Table 3 (source CREV).

In Emilia Romagna, the first serological monitorings were carried out in April and May 1999 in farms in the province of Forlì-Cesena which rear heavy chicken breeders. As in the case of light breeders, there was already a monthly plan for influenza serological auto-control. Twelve farms were monitored with a total of 124 samples, none of which were found positive in HI tests for influenza virus type A, subtype H7. During the same period, a monitoring programme was organised for poultry consignments sent for slaughter into the area from areas infected by H7N1 in Veneto and Lombardy. Five of the 49 flocks tested were found to be positive for H7N1 (10.2%): 4 virologically and only one serologically. After these results the Emilia Romagna Regional Veterinary Service banned the acceptance of poultry for slaughtering from "high risk areas"

to slaughterhouses in areas registered with a high density of avian farms. The "high risk areas" had been instituted by regional and ministerial regulations around the farms infected with influenza virus, but successively the law was rescinded after the adoption of control measures for avian influenza and certification of the consignments by the infected Regions. Following the isolation of an influenza virus H7N1 with low pathogenicity in a turkey farm in the province of Ravenna, all the farms connected epidemiologically were placed under supervision and controlled clinically and serologically. In the period from 5 August to 26 August, 24 farms were controlled with two serological checks at a 15-day interval for a total of 1091 samples examined, and 3 farms were found positive with 48 positive samples (Tab.4a). In these seropositive farms all the poultry present were destroyed even in the case of poultry kept together with infected groups but which resulted negative to serological and/or virological tests. In total, until 31/08/99, about 11,058 birds were killed, namely, 6015 turkeys, 5037 chickens and 6 guinea fowl (Tab.4b), from the 3 infected farms which all belonged to one owner and were situated within one square km in an area of low density poultry-industry. This was done voluntarily, under official control at the appropriate rendering plant.

Since 1/10/99 the Ministry of Health has adopted a serological monitoring programme in all the farms where there is European Union trade in eggs for hatching or one-day-old chicks by taking 20 blood samples from each farm at 15-day intervals. Until now 24 farms in 8 provinces of Emilia Romagna with a total of 480 samples have been tested under this monitoring programme, and all proved negative with respect to avian influenza.

In general, it is necessary to point out that the data held by the National Reference Centre is incomplete and is often not even consistent, which is reflected in the following description of the situation.

From a clinical and anatomopathological point of view in the official outbreaks, the following illustrative situations can be described:

1) Meat turkeys: normally there was respiratory distress and suffocation, with a mortality rate between 5% and 97% in relation to the age of the birds affected. The gravity of the lesions must be related to the mortality rate; and in some cases the pancreas was affected, which appeared haemorrhagic and hardened.
2) Turkeys breeders: the respiratory problems recorded above were present but slighter and were associated with a drop in egg production of 30% to 80%. The morbidity rate of the disease was close to 100% while the mortality rate varied from 5% to 20%.
3) Broilers: in some cases the infection was not apparent, while in others it was accompanied by anorexia and respiratory symptoms of moderate gravity with a mortality rate between 2% and 3%.

4) Chicken breeders: a drop in egg production of 5% - 20% was noted, associated with a cyanosis of the comb and wattles and a mortality rate between 3% and 8%.

In these animals the clinical cases of avian influenza were confirmed by the isolation of the virus and the subsequent identification of the subtype and characterisation of the pathogenicity. In other categories or species the diagnosis of the disease was carried out only by clinical and serological tests, but it is anyway interesting to record the epidemiological data relating to these cases:

a) Commercial layers: anorexia and depression was followed by a 3-10% drop in egg production with a peak of 30%; however, only in a few particular cases were these percentages recorded - for the most part the drop in egg production was 2-3 points lower than standard production levels.
b) Guinea fowl breeders: the morbidity rate was 100% and the mortality rate between 5% and 20% while there was a constant presence of conjunctivitis.

In Italy strong pressure is developing to permit the use of vaccine as a control measure, nevertheless until now this possibility has been rejected by the National Health Service. Recently it has been proposed to use strategic vaccination under the control of the official veterinarian in the reference farms sited in the affected area. In addition, the Public Veterinary Service will ensure a programme for reducing the poultry density, modernising the plants and establishing definite parameters to improve the poultry industry in order to enhance the quality of the Italian poultry products.

In conclusion, it can be said that low pathogenic avian influenza infection has led to notable economic losses in the Italian poultry industry and caused real financial difficulties in some businesses. Therefore, a revision of the current European community legislation on avian influenza is strongly urged so that appropriate policies for the obligatory eradication through stamping out can be adopted also for these particular, low pathogenic viruses H7 and H5 which are able to spread and mutate into pathotypes. Indeed, in Italy at present, the possibility of adopting a general stamping out would be considered by the various farms involved only if there was financial support from the local or national government. The success of this drastic approach to the problem has been confirmed by the progress of the disease in Emilia Romagna where the owner of the affected farm voluntarily and hastily destroyed all the animals present.

In relation to the potential transmission of this virus to man, the Health and Prevention Departments in the regions concerned have been advised to

programme voluntary sampling from subjects exposed to possible transmission, which was also done in the case of the H5N2 virus in Veneto during 1997.

Table 1. Italian outbreaks of low pathogenic avian influenza virus H7N1 during 1999

Region	Number of isolates	Species (%)	HA0 cleavage site (%)
Lombardia (07/04/99 to 06/08/99)	26	Turkeys (68%) Chickens (32%)	PEIPKGR*GLF (69.2%) PEVPKGR*GLF (30.8%)
Veneto (31/03/99 to 15/10/99)	29	Turkey (75%) Chicken (25%)	PEIPKGR*GLF (100%)
Emilia Romagna (03/08/99 to 05/10/99)	3	Turkey (66.6%) Chicken (33.3%)	PEVPKGR*GLF (100%)

Table 2. Serological monitoring program in the poultry farms of Lombardia Region during 1999 (March-October)

Species	Poultry farms monitored	Poultry farms positive %	Birds monitored	Birds monitored in positive poultry farms	Birds positive for H7	Birds positive %
Layers	175	5.7	3608	220	81	36.8
Pullets	31	0.0	600	0	0	0.0
Broilers	5	0.0	80	0	0	0.0
Heavy breeders	82	17.0	2007	359	280	77.9
Turkeys	21	57.7	360	220	128	58.1
Guinea fowl	3	0.0	34	0	0	0.0
Other species*	23	0.0	425	0	0	0.0
Total	340	10.6	7114	799	489	61.2

* Game birds and domestic ornamental fowls.

Table 3. Serological monitoring program in the poultry farms of Veneto Region during 1999 (from 06/08/99 to 15/11/99)

Species	Poultry farms monitored in the infected area			Poultry farms monitored in other areas		Total poultry farms monitored
	Tested	Positive*	%	Tested	Positive	
Turkey breeders	18	7	38.9	16	0	34
Meat turkeys	57	7	12.3	30	0	87
Breeders	29	4	13.8	22	0	51
Layers	20	0	0.0	53	0	73
Broilers	28	0	0.0	137	0	165
Other species	4	0	0.0	15	0	19

* One or more birds positive with titre >=1:16

Table 4a. Clinical and serological monitoring in the farms of Emilia Romagna Region during the period of August 1999

Poultry farms monitored	Poultry Farms positive	Birds monitored	Birds positive for H7
24	3	1091	48

Table 4b. Data about the voluntary depopulation in the three poultry farms positive in the monitoring programme in Emilia Romagna Region in August 1999

Species and number of birds	Serological detection	Virus isolation	Birds killed
Turkeys - 6015	positive	H7N1	6015
Pullets - 1407	positive	H7N1	1407
Broilers (Capon) – 3630	positive	Negative	3630
Guinea fowl - 6	not done	Negative	6
			Total: 11,058

LITHUANIA: REPORT FOR AVIAN INFLUENZA

Edvardas Kazeniauskas

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The Departments of Poultry Disease and Virology of the National Veterinary Laboratory carry out a general monitoring programme for avian influenza in Lithuania.

The monitoring programme has been in place for 10 years for all poultry farms. Birds of all ages are included and participation is obligatory. Assessment is made each spring. At other times investigations are carried out on request by veterinarians or in the case of disease.

There is no special monitoring programme for pigeons, but wild pigeons around large poultry farms are investigated in spring.

Investigations using serological tests for avian influenza and Newcastle disease have been carried out on wild ducks that fly across Lithuania in the spring. In 1988 to 1990 these were joint collaborations with the Institute of Poultry Disease, Sankt Petersberg and in 1994-1995 the national veterinary Laboratory alone. All tests were negative.

Avian influenza has never been detected in poultry in Lithuania by serological methods, clinical signs, post mortem examination or investigations of pathological materials sent to the National Veterinary Laboratory.

Lithuania is free from avian influenza.

COUNTRY REPORTS FOR 1999

NEWCASTLE DISEASE

Representatives of the following countries made statements of no Newcastle disease outbreaks during 1999:

Spain
Finland
Denmark
Ireland
Poland
Hungary
Slovak Republic
Slovenia
Czech Republic
Estonia
Bulgaria
Romania

NEWCASTLE DISEASE: SITUATION IN GREECE IN 1999

Vasiliki Rousi

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Epidemiology

During 1999 a total of eight suspected cases was investigated for ND, these included poultry, pigeons, ostriches and partridges, all were negative.

Diagnosis and control

For virus isolation and the detection of antibodies to ND virus the methods comply with Directive 92/66/EEC.

Since June 1998 the Ministry of Agriculture has been drawing up a contingency plan for the rapid and efficient eradication of ND, this has now been submitted to the EEC for approval.

Vaccination

Vaccination against ND is not compulsory for chickens and turkeys in Greece, but practically all flocks of broilers, layers and breeders are vaccinated with live or inactivated vaccines.

NEWCASTLE DISEASE - SITUATION IN PORTUGAL 1998/1999

Miguel Fevereiro and Teresa Fagulha

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Introduction

The Newcastle disease (ND) situation in Portugal is different from that observed in the early 90's. ND was absent in industrial poultry flocks during 1998 and is apparently under control in this sector. In contrast, the cases of ND reported last year were all in pigeons. Though vaccination is compulsory, it is not sure that all racing pigeons have been vaccinated. The presence of ND virus in these birds is a serious threat for the poultry industry, because the means to control trade and movement of such birds are far from being satisfactory.

ND virus isolations in 1998

In 1998 there were 7 isolates from 34 suspected cases. All viruses were isolated from pigeons, six from the Lisbon area and one from Estremoz-Alentejo.

Antigenic grouping of 1998 NDV isolates revealed two groups: P and C1.

The ICPI was determined for 5 isolates and ranged between 0.96 and 1.94. IVPI was 0.00 on two isolates tested.

The amino acid sequence at the F0 cleavage site of six isolates showed that all have the motif RQKRF, the minimum requirement for pathogenicity.

ND virus isolations in 1999

During the first 10 months of 1999 a total of 28 samples were submitted for NDV isolation. One PMV-1 was isolated from a clinical case in racing pigeons in the Lisbon area. This isolate was inhibited by the monoclonal antibody 617/161 (supplied by the EU reference laboratory) specific for the pigeon PMV-1.

Plaque formation on MDBK cells was done. The virus 4859/99 produced plaques in the absence of trypsin.

The amino acid sequence at the F0 cleavage site revealed the presence of multiple basic amino acids at the C-terminus of the F2 protein and a phenylalanine (F) at residue 117.

A second PPMV-1 was isolated in the beginning of November from a 5 month old pigeon. Biological and molecular characterisation are being carried out.

Table 1. Isolation of PMV-1 viruses in 1998

Isolate	Type of bird	Characteristics			
		ICPI(a)	IVPI(a)	mAb(a)	AA sequence (cleavage site)
4474/98	*pigeon*	0.96	0.00	P	SGG**GRQKRF**I
4638/98	*pigeon*	1.25	0.00	P	SGG**KRQKRF**I
6667/98	*pigeon*	1.34		P	SGG**KRQKRF**I
6717/98	*pigeon*	1.375		P	SGG**KRQKRF**I
8145/98	*pigeon*			P	SGG**RRQKRF**I
8742/98	*pigeon*	1.94		C1	
8893/98	*pigeon*			P	SGG**KRQKRF**I

Table 2 . Isolation of PMV-1 virus in 1999

Isolate	Bird	PF[(a)]	Amino acid sequence at F0 cleavage site
4859/99	pigeon	yes	SGG**RRQKRF**I

[(a)] Plaque formation on MDBK cells in absence of trypsin

NEWCASTLE DISEASE: SITUATION IN ITALY DURING 1998 AND 1999

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Introduction

Italy has a voluntary vaccination policy against Newcastle disease, therefore all turkey and broiler breeders and the majority of meat-type birds are vaccinated.

Investigations on Newcastle disease during 1998 and 1999:

1998

Non virulent strains
A total of 3 non virulent (vaccine) strains was isolated in 1998

Virulent strains
Table 1. Virulent ND virus isolations

Case investigated	Date	Province	Species	Flock	Result
116/AV/98	20.03.1998	Gorizia	chicken	Rural	PMV1, C1, ICPI 1.69
192/AV/98	25.05.1998	Pisa	pigeon	Free	PPMV1, P, ICPI 1.3
209/AV/98	04.06.1998	Parma	pigeon	Free	PPMV1, P, ICPI 1.23
290/AV/98	10.09.1998	Parma	pigeon	Free	PPMV1,P, ICPI 0.85

1999

Non virulent strains
A total of 2 non virulent strains was isolated in 1999 (one in chickens and one in turkeys)

Virulent strains
No virulent strains were isolated in 1999 from domestic poultry (excluding pigeons).

Table 2. Isolations of PPMV1 strains in 1999

Case investigated	Date	Province	Species	Flock	Result
1047/V99	12.03.1999	Parma	pigeon	Rural	PPMV1, ICPI 0.5
1884/V99	04.05.1999	Parma	pigeon	Rural	PPMV1, ICPI 0.7
3672/V99	07.10.1999	Parma	pigeon	Rural	PPMV1, ICPI 0.6
3993/V99	29.10.1999	L'Aquila	pigeon	Rural	PPMV1, ICPI 1.1

All viruses isolated were isolated in SPF eggs and were characterised according to EU directive 92/66/EEC. In our laboratory, identification, preliminary characterisation with mAbs (supplied by CVL) and virulence tests were performed on all isolates.

Of the ten virulent ND viruses isolated during the last two years, nine were pigeon paramyxovirus 1. It should be noted that the isolates obtained from outbreaks in northern Italy (Parma and Modena) have an ICPI, which is lower compared to the reference PPMV1 strains and to the isolate obtained from central Italy. A similar situation has been reported by Meulemans *et al.*1998, and it would be of great interest to investigate it further.

References:

Meulemans et al. 1998 “Newcastle disease situation in Belgium” Proceedings of the Fifth annual meetings of the national Newcastle and Avian Influenza Laboratories of countries of the European Union. Edited By D.J.Alexander

NEWCASTLE DISEASE: SITUATION IN FRANCE DURING OCTOBER 1998 TO OCTOBER 1999

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During the period under consideration, data collected from three diagnostic laboratories showed that a total of 17 suspected cases was investigated from poultry : chickens (7), turkeys (1), guinea fowl (1), pigeons and game birds (1) located in different departements (Aisne, Pas de Calais, Côte d'Or, Nord, Oise, Bas-Rhin, Nièvre, Yonne, Tarn, Côtes d'Armor, Gard, Essonne, Rhône, Charente).

Twelve cases were negative.

Only 5 cases were positive (Table 1). None of the PMV1 isolates from chickens was pathogenic. Of pigeon PMV1 isolates, one doesn't react with the mAb 161/617 even with an ICPI > 0.7. The European Reference Laboratory completed the characterisation of this virus by the immunoperoxidase binding test which revealed patterns identical to those of group E, i.e. a vaccine virus and due to the very low inhibition with mAb 7D4 it was concluded that this isolate is B1-like. The value of the ICPI has to be confirmed.

Table 1: Avian PMV1 isolated from October 1998 to October 1999 in France

Country (Departement)	Month Year	Species	History Case	Reaction with mAb 161/617	ICPI
Côte d'Or	Jan.	Racing Pigeon	Mortality	+	1,39
Pas de Calais	Apr.	Racing Pigeon	?	+	0,85
Aisne	May	Chicken	Mortality	nd	0,15-0,18
Rhône	May	Racing Pigeon	Mortality	-	1,40
Charente	Aug.	Chicken	?	nd	0,00

nd : Not done

NEWCASTLE DISEASE SITUATION IN BELGIUM 1999

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Newcastle disease virus was only isolated once from pigeons during the year 1999.

The virus had an ICPI of 0,67 whilst the cleavage site of the F protein was consistent with the motif of pathogenic ND viruses: RQKRF.

NEWCASTLE DISEASE: COUNTRY REPORT FOR THE NETHERLANDS

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Newcastle disease outbreak in exotic birds

A trader in Drachten, The Netherlands exported a total of 2691 exotic birds consisting of 175 common canaries, 543 assorted parakeets and 1973 assorted finches, to Montreal, Quebec, Canada on 26th November 1998. On arrival 24 birds were dead and 50/130 cockatiels died during quarantine. A haemagglutinating virus was isolated from the dead birds which was inhibited by APMV-1 but not APMV-2 or APMV-3 antiserum in haemagglutination inhibition tests and in ICPI tests gave a value of 1.6. The amino acids at the F0 cleavage site were RRQKR/F.

On the 21st December 1998 officers from the Veterinary Service visited the Dutch trader. A total of 5771 birds were housed in about 180 cages on the premises. Twenty live and apparently healthy birds, 12 dead birds (cockatiels) and 143 faeces samples were collected for virus isolation attempts, by inoculating eggs with pools of 10 samples. Embryo mortality occurred in eggs inoculated with 2/10 of the faeces pools and haemagglutination activity was detected in harvested allantoic fluid. The presence of virulent APMV-1 was confirmed by a positive reaction with extracted RNA in an RT-PCR test using primers specific for virulent NDV. The sequence at the cleavage site was RRQKR/F.

NEWCASTLE DISEASE - SITUATION IN GERMANY 1998 - 1999

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Vaccination against ND is compulsory for chickens and turkeys in Germany. Due to this policy Germany has been free of ND since April 1996.

Since all chickens and turkeys have vaccine-induced antibodies serological screenings for surveillance purposes are not feasible.

Therefore all suspected cases are investigated virologically.

In 1998 1232 cases were investigated in the regional diagnostic laboratories. Ninety-seven viruses were isolated and 50 of them were submitted to the National Reference Laboratory (Table 1). The isolates were typed by HI tests with polyclonal sera and monoclonal antibodies and tested for their pathogenicity.

Forty-three isolates were identified as PMV-1. Thirty-three of them were pigeon type PMV-1.

One velogenic NDV was obtained from a dead wild pigeon, but there was no evidence of an epizootic in further wild birds.

Eight lentogenic PMV-1 were isolated from chickens and one from a turkey. They all were viruses of the La Sota type since they reacted with the monoclonal antibody 7D4 in the HI test.

Three viruses from parakeets could be identified as PMV-3.

Four other apathogenic haemagglutinating isolates from turkeys and chickens were identified as PMV-2 by the EU Reference Laboratory.

In 1999 the regional laboratories in Germany carried out the following diagnostic activities for ND (Table 2).

25 808 sera from poultry and other birds were tested for antibodies to PMV-1. Most samples came from chickens and turkeys for controlling the antibody titre

after vaccination, but 356 sera of unvaccinated birds were tested because of suspicion of infection.

Attempts for virus isolation were made in 969 cases. The reason for this investigation was the suspicion of ND in 50 cases only, the other were performed for clearing up the cause of death, so to speak, as routine procedure.

Sixty haemagglutinating viruses were isolated and, up to now, 42 of them have been submitted to the National Reference Laboratory (Table 3).

Forty-one were identified as PMV-1.

There was no velogenic isolate found.

Twenty-nine isolates were characterised with monoclonal antibodies as pigeon type PMV-1. Most of them were isolated from pigeons, but a few came from other birds.

Twelve viruses proved to be lentogenic. All of them were viruses of the La Sota type since they reacted with mAb 7D4 in the HI test.

We identified one isolate from a pet bird as PMV-3.

For the evaluation of regional laboratories we organised a comparative test for virus isolation this year. Twenty-three laboratories from all Bundeslanders were involved.

Four samples of tissue material, two infected with PMV-1, one with influenza virus and one negative sample, were dispatched. The participants should treat the samples as if they originated from infected flocks. They should try to isolate and to identify the viruses.

The results of the comparative test were satisfactory.

Table 1. Virus isolates characterised by the National Reference Lab in 1998

No	Virus	Subtype	isolated from:
43	PMV-1	33 pigeon type	pigeons 27, chickens 3, canary 1, parrot 1, quail 1
		1 velogenic	wild pigeon
		9 lentogenic	chickens 8, turkey 1
3	PMV-3		parakeets
4	PMV-2		turkeys 2, chickens 2

Table 2. Diagnostic activities for ND in the Regional Laboratories in 1999

Kind of investigation:	virological	serological
Samples	969	25 808
Reason for investigation:		
Suspicion of ND	50	356
Cause of death	919	-
Vaccination control	-	25 452
Origin of samples		
chickens	408	21 946
turkeys	30	3 527
pigeons	298	280
ducks	68	14
geese	36	4
other poultry	9	4
parakeets/parrots	36	27
other pet birds	43	-
wild/zoo birds	41	6

Table 3. Virus isolates characterised by the National Reference Laboratory in 1999 up to 31/10/99

No	Virus	Subtype	isolated from:
41	PMV-1	29 pigeon type	pigeons 24, chicken 1, partridge 1, parrots 2, falcon 1
		12 lentogenic	chickens 7, pigeons 2, duck 1, raven 1, cockatoo 1
1	PMV-3		waxbill

NEWCASTLE DISEASE: COUNTRY REPORT FOR SWEDEN 1999

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Investigation

During 1999 five suspect cases (2 turkey flocks, 2 game bird farms with pheasants and partridges and one backyard flock with pigeons, chickens, pheasants and budgerigars) were investigated for ND virus in Sweden, all cases were negative.

Serological surveys

In Sweden, no NDV seropositivity is tolerated in poultry flocks, therefore a non vaccination policy for NDV is in effect. Active serological surveys for antibodies to NDV in Swedish poultry population are in place to preserve the status of an officially declared NDV infection-free country.

A total of 10,000 samples was tested for antibodies to ND-virus during 1999. No antibodies were detected.

Health monitoring in European cormorant

Three colonies of European cormorant, *Phalacrocorax carbo sinensis*, investigated in health monitoring projects were tested for antibodies to paramyxoviruses. Blood samples from seventy juvenile cormorants were used for serum analysis. In four samples a low reaction was detected with ELISA (SVANOVIR® , NDV- Blocking ELISA) but all samples were negative with HI-test. No clinical signs were observed in the cormorant population. No virus was isolated. The investigation will continue during 2000.

NEWCASTLE DISEASE: SITUATION IN GREAT BRITAIN 1999

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Investigations of Newcastle disease in poultry during 1999.

During 1999 a total of 15 suspected cases of Newcastle disease (ND) was investigated in poultry. No virus was isolated (Table 1).

Table 1. Newcastle disease investigations in poultry during 1992-1999.

	1992	1993	1994	1995	1996	1997	1998	1999
Cases investigated	17	16	22	11	44	107	12	15
NDV isolated	1	4	11	2	3	23	0	0
Confirmed cases	0	0	0	0	1	11	0	0

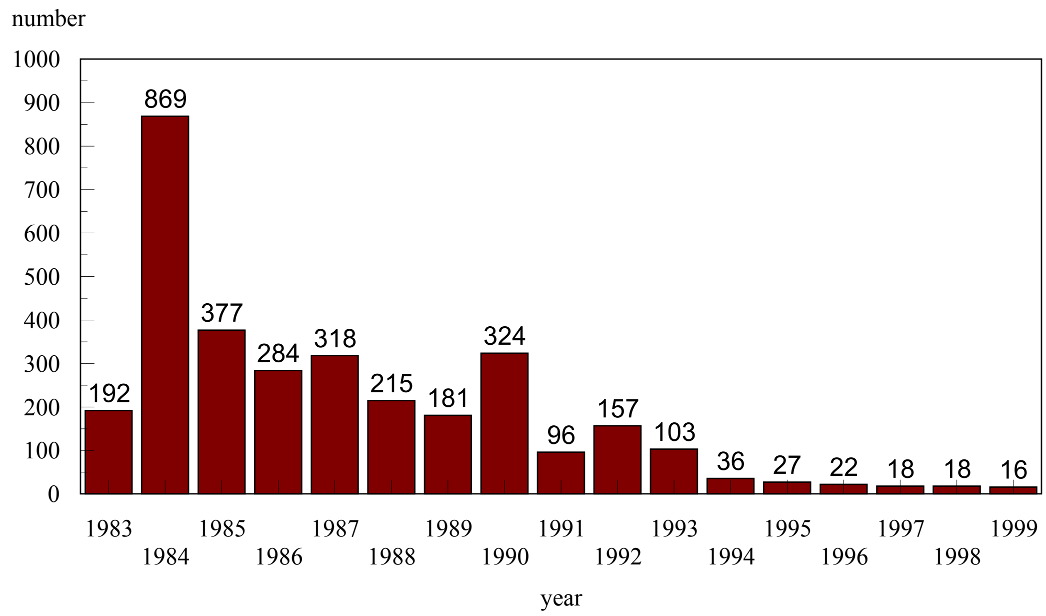
ND in racing pigeons

Infections of racing pigeons with the variant PPMV-1 virus are usually confirmed in Great Britain by virus isolation following investigations. The numbers of confirmed cases in Great Britain since the introduction of this variant virus in 1983 are shown in Figure 1. There were 16 confirmed cases in 1999, the lowest since the introduction of the virus. All were confirmed as the pigeon panzootic virus, but two isolates showed unusual mAb binding patterns in that they did not react with mAb 161/617. MAb 161/617 also failed to inhibit these viruses in haemagglutination inhibition tests.

ND in other birds

During 1999 there were 135 virus isolation attempts on birds dying in quarantine. No ND viruses were isolated, but two APMV-3 viruses, 3 reoviruses and two herpesviruses were obtained from various psittacine species and a rotavirus was isolated from a pigeon.

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



NEWCASTLE DISEASE: SITUATION IN AUSTRIA 1999

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During 1999 4 outbreaks of Newcastle disease in pigeons occurred in hobby flocks of two regions in Austria. The isolated PPMV 1 strains from the four holdings showed all an ICPI of about 1.4.

NEWCASTLE DISEASE: REPORT FOR NORWAY OCTOBER 1998 - OCTOBER 1999

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There has been no recorded outbreak of Newcastle disease during the report period, and all diagnostic testing for PMV-1 and PMV-1 antibodies on clinical and pathological indications has given negative results.

Twenty-four flocks in import isolation were tested serologically with negative results.

As a part of a programme for health certification and documentation samples from flocks on all parent poultry farms are routinely tested for PMV-1 antibodies.

During the 12 month period approximately 7100 samples representing 114 flocks were tested. Seroreactors were recorded in one single flock where seroconversion coincided with the blood sampling. No disease signs were recorded, and four other flocks on the farm remained antibody negative. Follow-up virus isolation attempts, also on samples from sentinel chicks, were negative, and the sentinels remained antibody negative. Testing of birds on other farms with a similar vaccination programme failed to indicate any contaminated vaccine as cause of the seroconversion in the single flock.

LITHUANIA: REPORT FOR NEWCASTLE DISEASE

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The Departments of Poultry Disease and Virology of the National Veterinary Laboratory carry out a general monitoring programme for Newcastle disease in Lithuania.

The monitoring programme has been in place for 12 years for all poultry farms. Birds of all ages are included and participation is obligatory. Assessment is made each spring. At other times, usually 2-3 times per year, veterinarians and poultry farms request investigations of antibody levels to evaluate vaccination. Other investigations for Newcastle disease are in the case of disease signs.

There is no special monitoring programme for pigeons, but wild pigeons around large poultry farms are investigated in spring.

Investigations using serological tests for avian influenza and Newcastle disease have been carried out on wild ducks that fly across Lithuania in the spring.

Newcastle disease has been detected on one occasion on one farm "Vievio paukštynas" in 1988. Clinical signs were observed in three poultry flocks of about 150,000 chickens 35-60 days old. Virus was isolated at the National Veterinary Laboratory of Lithuania. The virus was sent to the Institute of Poultry Disease of Sankt Petersburg and later at the Institute of Virology, Pokrow, Russia. The virus was described as mesogenic, but no pathogenicity indices were reported. An eradication policy of birds with clinical signs was carried out with surveillance of other clinically healthy flocks. Vaccination with La Sota was also implemented and this farm is the only one in Lithuania to have been using vaccination continuously since 1988. Vaccination of other poultry began on large farms in 1993 and all other farms in 1994.

Newcastle disease has never been detected since 1988 in poultry by serological methods, clinical signs, post mortem examination or investigations of pathological materials sent to the National Veterinary Laboratory.

Lithuania is free from Newcastle disease.

NEWCASTLE DISEASE AND AVIAN INFLUENZA SITUATION IN SWITZERLAND

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The disease situation concerning AI and ND in 1998 and 1999 in Switzerland is as follows:

AI no cases reported (last case in 1930)
ND last two cases in 1996 and 1998 (two small fancy breeder flocks)

Monitoring is done:

SEROLOGY:

AI no regular screening so far in commercial flocks
selective monitoring of fancy breeder flocks in 1999
(IDEXX-ELISA/919 blood samples from 45 flocks, 18 flocks with single positive results 3,2 %)

ND routine serology of 1% of Swiss bred poultry flocks by haemagglutination inhibition tests.
serological testing of 90 % of imported live poultry and psittacines

VIROLOGY:
annually 30-50 organ samples from all species of birds, including feral birds, are monitored (no isolates in 1998/1999)

COMPARATIVE TESTS FOR ANTIGEN IDENTIFICATION IN DIFFERENT NATIONAL LABORATORIES 1999

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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 avian 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:

1. *All laboratories should hold at least two antisera to H5 influenza A subtype prepared against viruses with different neuraminidase subtypes and at least two antisera to H7 influenza A subtype prepared against viruses with different neuraminidase subtypes.*
2. *All laboratories should hold PMV-3 antiserum.*
3. *Laboratories, especially those with incorrect results, should re-consider the panel of antisera maintained for the identification of notifiable avian viruses.*
4. *Another comparative antigen identification test should be done in 1999.*

These were endorsed at the Fifth Annual Meeting held in Austria in 1998 and the Community Reference Laboratory undertook to organise a test in 1999.

There were three basic objectives:

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

Materials and methods

Each National Laboratory was sent 6 unknown antigens with instructions to carry out identification of the antigens A-F 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. The antigens supplied and the minimum essential results were:-

ANTIGENS SUPPLIED WERE:-

Antigen	**Virus**	**Minimum essential result**
A	chicken/California/Yucaipa/56 - APMV-2	other*
B	A/turkey/Wisconsin/66 (H9N2)	other
C	turkey/England/1087/82 - APMV-3	APMV-3
D	A/turkey/England/69 (H3N2)	other
E	chicken/Ulster/2C/67 – APMV-1	APMV-1
F	A/African starling/England-Q/983/79 (H7N1)	H7

*i.e. not APMV-1, H5 or H7 influenza

Antigen C was an APMV-3 virus, these often show high levels of reaction with APMV-1 antisera, but it is important that National Laboratories are able to distinguish these from APMV-1 viruses as they are often present in turkeys and caged birds and may show ICPI values >0.7.

Antigens A, B and D were included as a paramyxovirus and two influenza viruses that were unrelated to the viruses that cause notifiable diseases.

Results

The results obtained and submitted to the Community Reference Laboratory are presented in Table 1. In this table the Laboratories coded 1-18 are those that took part in the 1998 exercise in the same order. These consist of all the National Laboratories plus Norway and the Reference Laboratory [which is coded 1]. The total is 18 because Belgium also represents Luxembourg, the United Kingdom is represented by two Laboratories, Great Britain and Northern Ireland, and in Italy the influenza and Newcastle disease Laboratories are different and results were received from each Laboratory. In addition the other countries invited to the 6th Meeting were asked to take part in the exercise, seven responded and submitted

results Bulgaria, Cyprus, Czech Republic, Hungary, Lithuania, Poland and Slovak Republic, they are numbers 19-25 in the order the results were received.

The results for each antigen and possible explanations for any discrepancies will be considered separately.

ANTIGEN A – APMV-2

Of the 25 laboratories taking part 12 identified the antigen correctly as APMV-2 [11/18 of the EU laboratories plus Norway]. A further six identified the antigen as a APMV of unknown serotype and six identified the antigen as a haemagglutinating virus not APMV-1, H5 or H7. Only one laboratory reported a serious misdiagnosis, laboratory 18 considered the virus to be influenza.

ANTIGEN B – H9N2

The minimum requirement that this was not APMV-1, H5 or H7 was achieved by 18/25 laboratories [12/18]. However, in two laboratories [21 and 25] this was by default as they were unable to identify the haemagglutinating agent and in a further 7 laboratories [3/18] the identification of "influenza" was by varying degrees of reliability. Nine laboratories [9/18] made a positive correct identification of H9 subtype influenza. One laboratory [10] failed to detect HA activity. One laboratory [15] reported the antigen to be of H2 subtype, almost certainly due to a high cross-reaction with H2N2 antisera, but nevertheless an incorrect result. One laboratory reported the antigen to be APMV-3! Four of the 25 laboratories [3/18] made the wholly incorrect identification of the antigen as H5. None of these gave details of the antisera used, but it would seem likely that this was the result of cross-reaction with antisera against an H5N2 virus.

ANTIGEN C - APMV-3

Only one laboratory produced the wholly incorrect result of identifying this antigen as APMV-1, presumably due the relatively high cross-reaction between APMV-3 and APMV-1 serotypes. Of the other laboratories, four [2, 5, 20 and 25] did not identify the antigen fully, none of these laboratories used APMV-3 antiserum. Twenty of the 25 laboratories [16/18] made the correct identification of APMV-3.

ANTIGEN D – H3N2

As with antigen B, the other non-H5 or H7 influenza virus, antigen D caused some problems in identifying tests. The batch of freeze-dried H3N2 antigen sent to laboratories 19-25 was different to that sent to laboratories 1-18, it was not of good quality and would have been of low titre. For this reason the results produced by 19-25 for this antigen should be ignored, but it is worth noting that laboratory 19 reported it to be inhibited by both H5 and H7 antisera. Of the remaining laboratories 9/18 gave the correct identification of H3 and a further 5 gave the minimum result of not APMV-1, H5 or H7. The four remaining

laboratories gave results that were wrong. Laboratory 7 identified the antigen as H1 and laboratory 16 as H2, the latter presumably due cross-reaction with H2N2 antiserum as a result of the shared N antigen. The remaining laboratories, 12 and 18 identified antigen D as H5, again this wholly wrong result was probably the result of cross-reaction due to a common neuraminidase with the virus used to prepare the antiserum used.

ANTIGEN E –APMV-1

This antigen was chicken/Ulster/2C/67, the EU recommended antigen for APMV-1 in HI tests. It should have produced the least problem in identification tests of the six antigens. The correct result was obtained by 22/25 laboratories. Three laboratories failed to identify the antigen, 17 reported it as an APMV-9 virus, 18 as APMV-3 and 20 was unable to identify the antigen.

ANTIGEN F - H7N1

This antigen should have been as straightforward as antigen E, but four laboratories failed to identify it as H7. Three laboratories [22, 24 and 25] were unable to identify the antigen and the fourth, [18] reported it as APMV-1.

GENERAL

Laboratories 1-18 are the same as those that took part in the 1998 exercise and this allows some comparisons to be made, these are summarised:

Number of laboratories that:-	1998	1999
1. Fully and correctly identified all antigens	2	6
2. Obtained at least minimum essential results with all antigens	10	6
3. Had one unacceptable[1] result	2	5
4. Had more than one unacceptable result	4	1

[1]an unacceptable result is one where an H5, H7 or APMV-1 virus is not identified or typed as something else or another antigen is typed as H5, H7 or APMV-1.

These results suggest a very slight improvement in overall performance. Six laboratories fully typed all the antigens compared to 2 in 1998 and only one had more than one unacceptable results compare to 4 in 1998. However, the same number, 6, laboratories failed to produce completely acceptable results. The narrowness of the improvement is also reflected in the performances of the individual laboratories since within the four categories 7 showed an improved performance, 6 a worse performance and five remained the same.

Discussion

The functions of the National Laboratories are not specified in great detail in the Directives on control of Newcastle disease and avian influenza. However,

Directive 92/66/EEC does state in Article 14: *"1. Member States shall insure that in each Member State there is designated: (a) a national laboratory at which facilities and expert personnel shall be maintained to permit full antigenic and biological typing of Newcastle disease virus......."* and Directive 92/40/EEC states in Article 14: *"1. Member States shall insure that in each Member State there is designated: (a) a national laboratory at which facilities and expert personnel shall be maintained to permit assessment of pathogenicity of influenza isolates .... and identification of influenza A viruses of H5 or H7 subtypes;"*.

Implicit in the minimum requirement is a requirement that National Laboratories do not identify other viruses as those that require notification or further attention and in this aspect there was no overall improvement.

Recommendations

1. Laboratories should ensure they conform to the recommendations made in 1998 [see introduction].
2. Another antigen identification test should take place in 2000.

Reference

Alexander D.J. & Manvell R.J. (1999). Comparative tests for antigen identification in different EU National Laboratories. Proceedings of the Joint Fifth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Vienna 1998, pp 71-77.

Table 1. Results of comparative tests for antigen identification.

Lab	A	B	C	D	E	F
1*	APMV-2	H9N2	APMV-3	H3N2	APMV-1	H7N1
2	APMV-2	H9	APMV-3?	H3	APMV-1	H7
3	APMV-?	**H5**	APMV-3	FLU?	APMV-1	H7
4	APMV-?	FLU?	APMV-3	?	APMV-1	H7
5	APMV-?	FLU[N2]	APMV-?	?	APMV-1	H7
6	APMV-2	H9	APMV-3	H3	APMV-1	H7
7	APMV-2	H9	APMV-3	FLU-H1	APMV-1	H7
8	APMV-2	H9	APMV-3	H3	APMV-1	H7
9	APMV-2	H9	APMV-3	H3	APMV-1	H7
10	APMV-?	no HA	APMV-3	?	APMV-1	H7
11	?	**H5**	APMV-3	?	APMV-1	H7
12	APMV-2	FLU	APMV-3	**H5**	APMV-1	H7
13	APMV-2	H9	APMV-3	H3	APMV-1	H7
14	APMV-2	H9(N2?)	APMV-3	H3(N2?)	APMV-1	H7
15	APMV-2	H2	APMV-3	H3	APMV-1	H7
16	?	**H5**	APMV-3	H2	APMV-1	H7
17	APMV-2	H9(N2?)	APMV-3	H3	**APMV-9**	H7
18	FLU	APMV-3	APMV-3	**H5**	**APMV-3**	**APMV-1**
19	?	**H5**	**APMV-1**	**H5/H7**	APMV-1	H7
20	?	FLU	?		?	H7
21	?	?	APMV-3		APMV-1	H7
22	APMV?	FLU	APMV-3	?	APMV-1	**FLU**
23	APMV?	FLU	APMV-3	?	APMV-1	H7
24	APMV-2	FLU	APMV-3	?	APMV-1	**FLU**
25	?	?	?		APMV-1	**?**

*results obtained by the Community reference Laboratory

ORIGINAL CONTRIBUTIONS

ABILITY OF NP- AND M- BASED RT-PCR TESTS TO DETECT TYPE A AVIAN INFLUENZA VIRUSES. WITH RESPECT TO THE STANDARDIZATION OF DIAGNOSTIC PROCEDURES.

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According to the EU definition of highly pathogenic avian influenza, as given in council directive 92/40/EEC annex III, molecular methods are authorised to characterise the cleavage site of H5 and H7 avian influenza viruses (AIV). In fact, several H5 and H7 HA (haemagglutinin) gene-based RT-PCR tests have been reported in the literature, using different sets of primers. However, no molecular methods are described in directive 92/40/EEC and no standardised and reference molecular methods have been officially proposed. Since the HA gene exhibits a higher evolution rate in comparison with other AIV genes, it should be advisable, in order to avoid false negative results - due to possible mutations in the viral gene area where the 3' end(s) of the HA primer(s) is (are) supposed to hybridize-, to use a polyvalent RT-PCR test able to detect all type A viruses.

Since comparison of consensus Eurasian and north American AIV nucleotide sequences have shown 94.8 % and 92.9 % homology for M and NP genes respectively (Suarez, 1999), these genes appear as potential candidates to target in the polyvalent RT-PCR test to design.

The purpose of the present paper is to evaluate the polyvalence (for AIV detection applications) of three RT-PCR tests : two NP- and one M- based.

Materials and Methods

Viruses

32 AIV strains (belonging either to the Eurasian lineage or to the North American lineage) were analysed. They represented all 15 HA subtypes and 8 of the 9 NA subtypes so far reported. A representative of N8 subtype being missing. 16/32 and 13/32 had been received from Dr D.J. Alexander (VLA, CVL, Weybridge, GB) and Dr M.L. Perdue (Southeast Poultry Research Laboratory, Athens, Georgia, USA) respectively, whereas 3 other strains had been isolated in France and their identity confirmed by the European Reference Laboratory. In order to check for specificity, 2 vaccinal Newcastle disease virus (NDV) strains: HB1 and La Sota, were analysed. All these strains were grown in the allantoic cavity of SPF eggs.

The RNA from each infective allantoic fluid was extracted using the Rneasy mini kit (Quiagen) according to the recommendations of the manufacturer.

Primers

Their sequences are given in Table 1. Primer AIV/all 8+ (M.L. Perdue personal communication) is complementary to the 3'end consensus sequence of every 8 segment of the RNA template. The other six primers were homologous to the consensus sequence of either NP or M gene as defined following the multialignment of respectively 15 and 20 avian influenza sequences from NCBI data bank representing strains isolated over 55 and 90 years respectively. In addition primers targeting the M gene were adapted from Suen et al. 1997.

cDNA synthesis

For cDNA synthesis, the same primer AIV/all 8+ was used irrespective of the RNA template and of the PCR test to be performed later on. The reverse transcriptase Superscript (Gibco BRL) was employed according to the specifications of the supplier.

The NDV RNAs were similarly processed.

PCR tests

Two sets of primers targeting the NP gene and one set targeting the M gene were used. The lengths of the expected amplified products are given in Table 1. The NP-based RT-PCR tests are further referred to as NP 626 and NP147 with reference to the length of the respective PCR products .

PCR tests were performed in a final volume of 20 µl using dNTPs (Perkin Elmer) (1,25 mM of each), $MgCl_2$ (Perkin Elmer) (2 mM), AmpliTaq Gold (Perkin Elmer) (1unit), specific primers (20 pmoles for each). Several dilutions of each cDNA were analysed. For NP-based PCR the following programme was run : 95°C for 12 min then 30 cycles of 95°C for 20 sec, 53°C for 30 sec, 72°C for 30 sec, and a final elongation at 72°C for 6 min. For M-based PCR, the same programme was applied but the elongation time that was 90 sec at each cycle and 12 min for final elongation. A GeneAmp 2400 (Perkin Elmer, Biosystems) was employed.

Automated sequencing of the PCR products

In order to confirm their specificity, a few PCR products (as detailed in results) were sequenced after having been purified from agarose gel using either Quiaquick gel extraction kit (QUIAGEN) or Geneclean (Bio101) according to the recommendations of the supplier. The purified PCR products were processed by the dye terminator method using the PCR primers listed in table 1, the ABI PRISM TM Dye terminator cycle sequencing kit (Applied Biosystems) and the DNA sequencer model 373A (Applied Biosystems) according to the recommendations of this supplier.

RESULTS

The results are summarised in Table 2.

The two NP-based RT-PCR tests were not applied to all 32 strains listed since they did not detect 100% of the strains investigated for screening. However, sequencing of 3 different NP 144 PCR products gave 87 to 93 % identity with the consensus NP sequence (mentioned above in chapter « primer »). No NP 626 PCR products were sequenced.

Only the M-based test was able to detect all 32 AIV strains analysed whereas NDV strains were not detected. As shown in Fig.1, two bands with the expected size (944 and 256 bp) were obtained using this test. Sequencing of the upper band gave approximate data with about 12-13% indeterminations since only the 5' part of either strand could be analysed using primers M31 and M944r only. In spite of this, sequencing of 5 different M PCR products exhibited at least 70-75 % homology with the M consensus sequence mentioned in the same chapter « primer ». Sequencing of the lower band was performed for only one PCR product. It showed that this fragment was overlapping the 944 bp fragment by being 5'coterminal for its 243 first nucleotides and 3' co-terminal for its 13 last nucleotides.

DISCUSSION

Only the present M-based RT-PCR test displays the ability to detect 32 out of 32 AIV strains analysed, the NP-based RT-PCR tests were not polyvalent enough for purpose detection. So the discussion will be focused on the M test.

A multiplex RT-PCR designed for the differentiation of AIV and NDV and employing M primers among others, was described by Suarez (1997). The latter test, which amplified a 224 bp fragment located at the 5' end of the M cDNA gene, was previously shown able to detect all 15 different AIV isolates analysed representing all known HA subtypes. Our RT-PCR M test also detects strains representing all 15 HA subtypes and at least 8 NA subtypes, since we have not had the opportunity to test N8 isolates.

However complementary investigations (data not shown) on test M revealed that inactivation of the virus renders the present test ineffective.

As cited by Suen et al (1997), resistance to amantadine is conferred by a single amino acid substitution at one of the five critical sites (position 26, 27, 30, 31 or 34) of the M2 protein which corresponds to nucleotides 801-806, 813-818, 825-827 of the M2 gene. Thus our M-based RT-PCR test could be useful to predict the amantadine resistance of a given strain in case reassortants viruses with risk for public health emerge.

The lower band of our M PCR product exhibits the expected size for the spliced gene (256 bp) since, according to Kingsbury (1990), the M2 RNAm possesses its first 51 nucleotides co-terminal with the M1 RNAm and places

nucleotide 51 adjacent to nucleotide 740. In fact, sequencing of the lower band, reveals that this PCR product corresponds to the 5' extremity of fragment (944 bp) without any splicing. In fact primer AIV 954r also hybridizes at its 3' end with nts 266-273 of the M gene. Consequently this M-based RT-PCR test will not be suitable to demonstrate transcription activity for further pathogeny studies. For that goal, a new reverse primer should be designed.

We have not checked experimentally whether the specificity of our M-based RT-PCR test was type A Influenza virus restricted by including type B virus strains ; however sequence analysis of 10 type B Influenza virus strains did not reveal mismatch of more then 5 nts at the 3' end of our type A M primers. So, even in case these partial hybridizations took place and were sufficient for amplification, the size of the resulting eventual PCR products would be quite different from the pattern presently observed.

It should also be worthwhile to evaluate the sensitivity of our present M-based RT-PCR test with the view of either quantitative tests -for instance for measurement of viral excretion- or with the view of detection tests performed directly on suspicious samples.

ACKNOWLEDGEMENTS

The authors are very grateful to D.J. Alexander (VLA, CVL, Weybridge, GB) and Dr M.L. Perdue (Southeast Poultry Research Laboratory, Athens, Georgia, USA) for providing the avian influenza viruses as well as to J. Lamande and M.O. Le Bras (AFSSA Ploufragan) for their excellent technical collaboration in growing viruses and to J. Rousset (AFSSA Ploufragan) for his participation in M-based RT-PCR evaluation. This work was supported by FEDER allowance.

REFERENCES

1-Kingsbury D.W., 1990. Orthomyxoviridae and their replication. In: Virology Fields B.N., Knipe D.M. et al edt, 2nd edt, Raven Press Ltd, New York, pp. 1075-1089.

2- Suarez D.L.,1997. Molecular diagnostic techniques: can we identify influenza viruses, differentiate subtypes and determine pathogenicity potential of viruses by RT-PCR. In: Proceedings of the fourth international symposium on avian influenza, Athens Georgia USA, May 29-31, 1997, pp 318-325.

3- Suarez D.L., 1999. Evolution of avian influenza viruses. In: Abstracts of the symposium on animal influenza viruses. ESVV. Gent, Belgium, May 16-18, 1999, pp 44.

4-Suen P.C., Tsao K.C., Wu T.L., Ning H.C., Liu T.Z. and Shih S.R., 1997. Reverse transcriptase-polymerase chain reaction for amantadine susceptibility testing of influenza viruses. Biotechnology Techniques, 11, 873-878.

Table 1. Primers and their use

Name	Sequence[a]	Use	Position
AIV/all 8+	ACG CGT GAT CAG CAA AAG CAG	cDNA synthesis	1-11[b1b2]
AIV NP 258	TGA TGA AAG GAG RAA YAA ATA	PCR NP 626[c]	258-278[b1]
AIV NP 863r	CAC AGG CAG GCA RGC ARG AYY		883-863[b1]
AIV NP 866	CCT GCT TGC CTG CYT GYG TRT	PCR NP 147[c]	866-886[b1]
AIV NP 992r	CTG GAT TCT CAT TTG RTC KYA		1012-992[b1]
AIV M 31	TCT TCT AAC CGA GGT CGA AAC	PCR M 944[d] 256[d]	31-51[b2]
AIV M 954r	GTC AAC ATC CAC AGC AYT YTG		974-954[b2]

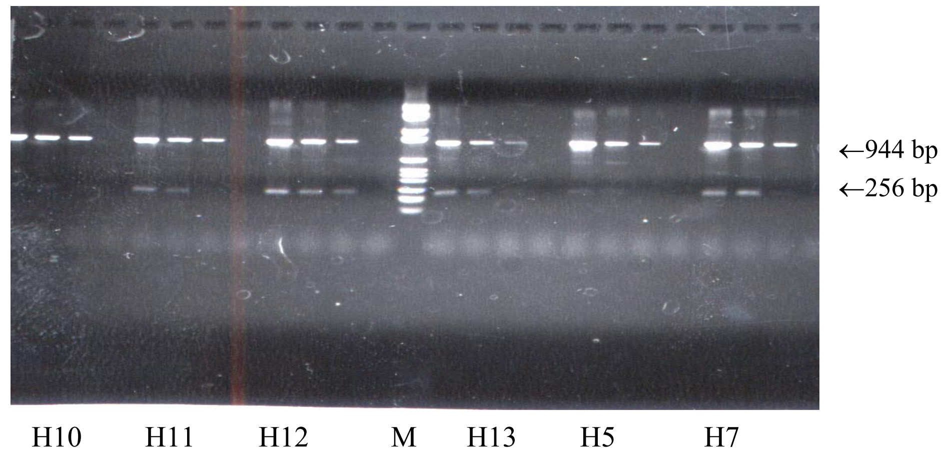
(a) The part complementary to the 3' end RNA template of each segment is underlined.
(b1) Position as referred to sequence of gene NP from AIV strain A/Duck/Australia/749/80 H1N1 (Accession N° M63783).
(b2) Position as referred to sequence of gene M from AIV strain A/FPV/Weybridge H7N7 (Accession N° L37797)
(c) Length of the expected PCR products.
(d) The gene being spliced (see the text) to give the M2 RNAm, two PCR products may be obtained exhibiting the lengths mentioned.

Table 2. Ability of 3 RT-PCR tests to specifically detect AIV

Viral strains		RT-PCR tests NP 626 bp	NP 147 bp	M
AIV	investigated	23*	15	32
	detected	20	12	32
aPMV1	investigated	ND	ND	2
	detected	ND	ND	0

* Number of strains

Figure 1. Detection of AIV using M-based RT-PCR test



H = HA subtypes

M = DNA marker

A PCR-BASED SCREENING ASSAY TO DETECT ALL KNOWN SUBTYPES OF AI; COMPARISON OF THE SENSITIVITY OF THIS SCREENING METHOD WITH PROPAGATION IN EGGS.

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Summary

The aim of this study was to set up a rapid and sensitive PCR method for screening specimens for the presence of phenotypically and genotypically diverse influenza A viruses. To this end, we have designed a primer-set for PCR-based detection of influenza A viruses that was validated with a panel of influenza A virus strains representing all known HA and NA subtypes obtained from a variety of host species and from different geographical locations. The efficacy of this PCR-based screening of samples from avian and human origin was compared with classical isolation of influenza A virus in embryonated chicken eggs or mammalian cell culture. We conclude that this PCR, based on the detection of gene segment 7 of influenza A virus, is fast, sensitive and specific and suitable for all genetic variants of influenza A virus known to date.

Results

Design of oligonucleotides for PCR detection of influenza A viruses.

Avian and mammalian influenza A virus nucleotide sequences available from the influenza sequence database (http://www.flu.lanl.gov) were compared to previously described primer-sets Mx1 and Mx2 (2), Fam1 and Fam2 (1) and NS486C and NS637R (3) to analyze their potential for the detection of genetically diverse influenza A viruses. Although each of the primer sequences was based on a relatively conserved domain of gene segments 7 and 8 of influenza A virus, considerable heterogeneity was observed for each of the oligonucleotide sets, including heterogeneity at the 3' ends of the oligonucleotides (4). We designed new primer-sets based on segment 7 of influenza A virus, which is relatively conserved as compared to the other segments. Within the M1 coding sequence of gene segment 7, several regions were identified that are relatively conserved among influenza A virus strains obtained from a variety of host species and from different geographical regions. Oligonucleotides M52C (nucleotide positions 32-52), M93C (71-93) and M253R (253-276) were designed based on these conserved regions of the influenza A virus genome.

Sensitivity and specificity of influenza A virus PCR
RNA was isolated from 4 µl allantoic fluid, containing influenza A viruses of all HA subtypes, and used for amplification by PCR with primer-set M52C-M253R. For each of the virus strains tested, a band of 244 basepairs was amplified, which was easily visualized on a 1 % agarose gel stained with ethidium-bromide (Fig 1). Hybridization of dot-blots with the internal biotinylated oligonucleotide probe M93C also resulted in clear signals for each of the influenza A virus strains tested. Comparison of the sensitivity of this PCR procedure to isolation of human influenza A virus in mammalian cell cultures revealed that PCR is up to 100-fold more sensitive than virus isolation in MDCK and tMK cells (4).

To test the specificity of our PCR primers, RNA was isolated from 0.2 ml of virus stocks (10^4-10^6 TCID50/ml) containing either influenza B virus, human respiratory syncytial virus (HRSV), parainfluenza virus type 1, 2, 3 and 4 (PIV-1, 2, 3, 4), simian parainfluenza virus type 5 (SV5), Newcastle disease virus (NDV), mumps, measles or Sendai virus. Upon agarose gel electrophoresis, weak bands and smears of bands, ranging from 150 to 400 basepairs in length, were observed after PCR amplification of some of the virus samples (PIV1, 2 and 3, NDV, mumps and influenza B virus), presumably as a result of non-specific amplification of the high levels of viral RNA present in these samples. However, upon hybridization of dot-blots with the biotinylated oligonucleotide M93C, all RNA virus samples except for influenza A virus, were negative (Fig. 2).

Detection of influenza A virus in bird samples
We next tested the suitability of the PCR for avian influenza A virus screening of cloacal swabs and droppings from a number of bird species. Because PCR screening appeared to be up to 100-fold more sensitive than virus isolation, and to reduce cost and workload, the number of RNA isolations and PCR analyses were reduced by making pools of 5 samples each (40 µl per sample). From 235 pools of samples representing 1175 individual specimens, 19 revealed the presence of influenza A virus upon RNA isolation, PCR and Southern or dot-blot hybridization (the analysis of 38 of these pools is shown in Fig. 3). RNA was then isolated from each of the individual samples present in these 19 pools, revealing that each pool contained a single positive bird sample except for one pool, which contained two positive samples.

Each of the 20 positive individual samples were used to inoculate 2 to 4 embryonated chicken eggs, from which the allantoic fluids were collected, pooled and inoculated a second time in duplicate in embryonated chicken eggs (blind passage). From 15 out of 20 PCR-positive samples we were able to isolate influenza A virus in eggs. From the other 5 samples, which appeared to contain less virus as judged by the intensity of signals on dot-blots (e.g. lanes 36, 44, Fig. 3), no influenza A virus could be isolated even upon blind passage in embryonated chicken eggs.

To test the possibility that the PCR analysis would give false negative results as compared to virus isolation in eggs, 243 individual PCR-negative cloacal swabs and dropping samples were inoculated in 2 to 4 embryonated chicken eggs each, followed by a blind passage of the pooled allantoic fluids in duplicate. We were unable to isolate influenza A virus from these PCR-negative samples, indicating that no false negative results were obtained by PCR analysis. Inoculation of tMK and MDCK cell cultures with 212 random PCR-negative individual bird samples also did not reveal additional influenza A virus-positive samples. In fact, these cell lines were found to be less susceptible to avian influenza A virus as compared to embryonated chicken eggs.

Procedure

Oligonucleotide sequences: M52C (5'- CTT CTA ACC GAG GTC GAA ACG – 3'), M253R (5'- AGG GCA TTT TGG ACA AAG/T CGT CTA -3'), Bio-M93C (5'- CCG TCA GGC CCC CTC AAA GCC GA –3') .

RNA was isolated using a high pure RNA isolation kit (Roche Molecular Biochemicals) according to the instructions from the manufacturer, with minor modifications. 0.2 ml sample was homogenized by vortexing and subsequently lysed with 0.4 ml lysis/binding buffer to which poly-A (Roche Molecular Biochemicals) was added as a carrier to 1 µg/ml. After binding to the column, Dnase I digestion and washing, the RNA was eluted in 50 µl nuclease-free double-distilled water preheated at 80°C.

The reverse transcription (RT) and PCR reactions were optimized with respect to enzymes, primer-sets, and concentrations of reagents as well as cycling parameters. Samples were amplified in a one-step RT-PCR in 25 µl final volume, containing 50 mM Tris.HCl pH 8.5, 50 mM NaCl, 7 mM $MgCl_2$, 2 mM DTT, 1 mM each dNTP, 0.4 µM each oligonucleotide, 2.5 U recombinant RNAsin, 10 U AMV reverse transcriptase, 2.5 U Ampli-Taq DNA polymerase (all enzymes from Promega Benelux B.V. Leiden, The Netherlands) and 5 µl RNA. Thermo-cycling was performed in an MJ PTC-200 apparatus using the following cycling conditions: 30 min. at 42°C, 4 min. at 95°C once and 1 min. at 95°C, 1 min. at 45°C, 3 min. at 72°C repeated 40 times. Each reaction was analyzed by agarose gel electrophoresis and ethidium-bromide staining (10 µl/sample) or dot-blot (5 µl/sample) or Southern blot hybridization.

For dot-blot hybridization, five µl of the PCR samples were incubated for 5 min. at room temperature with 45 µl 10 mM Tris.HCl pH 8.0, 1 mM EDTA and 50 µl 1 M NaOH for denaturation. Samples were transferred to prewetted Hybond N^+ membranes using a dot-blot apparatus while applying vacuum. Samples were then treated for 3 min. with 0.1 ml 1 M Tris.HCl pH8.0, after which vacuum was applied again for 10 sec, and the membrane removed from the apparatus. Blots were washed three times for 10 min. with 0.3 M NaCl, 30 mM Na-citrate pH 7, dried, and stored at 4°C. Blots were prehybridized for 5 min. at 55°C in 2 x SSPE (0.3 M NaCl, 20 mM NaH_2PO_4, 2 mM EDTA, pH 7.4)

and 0.1 % SDS, after which biotinylated oligonucleotide probe Bio-M93C was added to 2 pmol/ml and hybridization was continued for 45 min. at 55°C. Blots were washed twice for 10 min. at 55°C with hybridization buffer, transferred to 2 x SSPE with 0.5 % SDS after which streptavidin-peroxidase (Roche Molecular Biochemicals) was added to 0.125 U/ml and incubated for 45 min. at 42°C. Blots were washed 10 min. at 42°C in 2 x SSPE, 0.5 %SDS, 10 min. at 42°C in 2 x SSPE, 0.1 % SDS and 10 min. at room temperature in 2 x SSPE, after which the samples were visualized using ECL detection reagents and exposure to hyperfilm (Amersham Pharmacia Biotech Benelux, Roosendaal, The Netherlands) for 5 to 60 seconds.

Conclusion

Taken together, our data indicate that the newly designed PCR offers a more sensitive and faster tool for the diagnosis of human influenza A virus infection than virus isolation. Because of the better matching primers it can be expected that for the detection of animal influenza A viruses this PCR is also more suitable than previous PCR protocols (1-4).

References

1. Atmar, R.L. et al., J. Clin. Microbiol. 34:2604-6, 1996.
2. Cherian, T. et al. J. Clin. Microbiol. 32:623-8, 1994.
3. Claas, E.C. et al., J. Virol. Methods 39:1-13, 1992.
4. Fouchier, R.A.M. et al., Manuscript submitted.

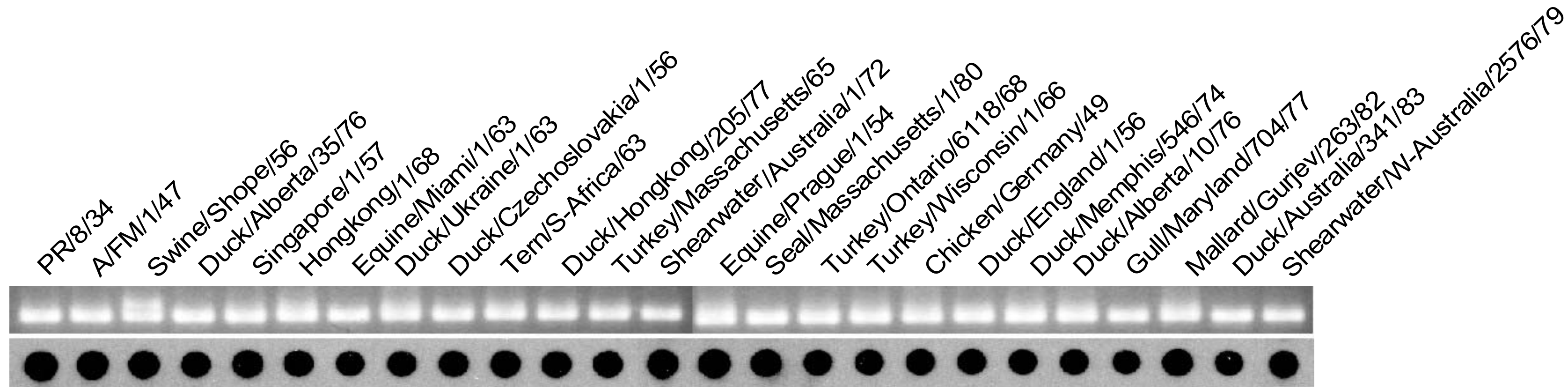

Figure 1. *PCR analysis using a representative panel of influenza A viruses, representing all HA and NA subtypes, originating from different hosts and geographical locations. RNA was isolated from influenza A viruses grown in embryonated chicken eggs, followed by PCR analysis and agarose gel electrophoresis (top panel) or dot-blot analysis (bottom panel).*

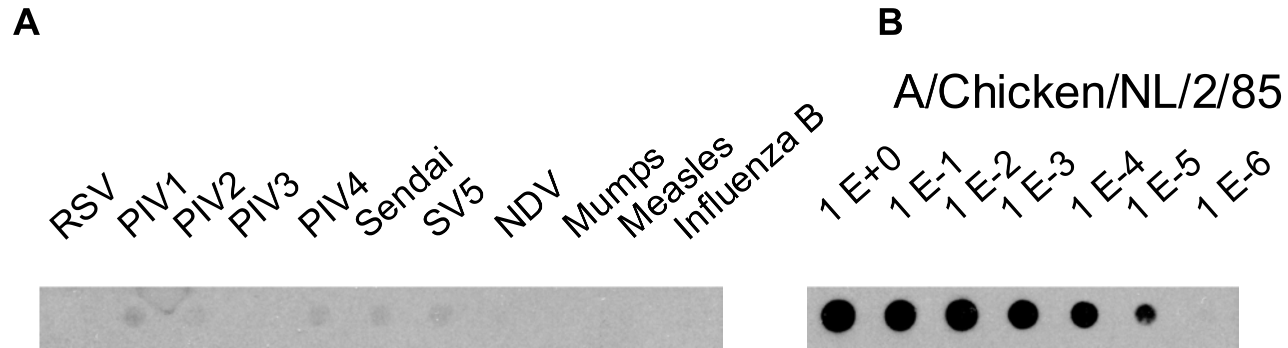

Figure 2. *Specificity of detection of influenza A virus RNA by PCR. RNA was isolated from virus stocks and used for PCR analysis and subsequent dot-blot hybridization. (A) A panel of RNA viruses (titers ranging from 10^4 to 10^6 TCID50/ml) (B) Ten-fold serial dilutions of influenza virus A/Chicken/Netherlands/2/85 (titer: 10^4 TCID50/ml).*

Figure 3. *PCR-based detection of influenza A virus in a representative set of avian cloacal swabs and droppings. RNA was isolated from 0.2 ml of 38 pooled samples, each consisting of 5 individual bird samples, and used for PCR and Southern blot analysis.Pc; positive control, Nc; negative control, Dc; duck cloacal swab, Gd; Goose dropping, Gc; Goose cloacal swab. Each of the positive pools was found to contain a single positive individual bird sample. Virus was isolated in embryonated chicken eggs from all positive samples except the two with faint hybridization signals in the bottom two pannels.*

THE LOW PATHOGENICITY AVIAN INFLUENZA (H7N1) EPIDEMIC IN THE VENETO REGION, ITALY

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Introduction

From the 29th of March to the end of October 1999, one hundred and sixty-three outbreaks of LPAI were diagnosed in the Veneto and Lombardia regions, which are located in the northern part of Italy; the most affected region being Veneto. The outbreaks affected several avian species including turkey and chicken breeders and broilers, layers and guinea fowl breeders and meat birds. The highest number of outbreaks was diagnosed in turkeys, namely 121 outbreaks in broilers and six in breeders. Nineteen outbreaks were reported in broiler breeders and only three in broiler chickens. A total of 12 outbreaks were diagnosed in layers, one in guinea fowl breeders and one in meat type guinea fowl. In this paper the results of the clinical, post-mortem and laboratory investigations carried out during the epidemic are presented.

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.

Serological and virological investigations were performed in accordance with the guidelines indicated in EU Directive 92/40/EEC (CEC, 1992). The haemagglutinin (H) and neuraminidase (N) subtypes of influenza A isolates were determined using polyclonal chicken antisera as described by Alexander and Spackman (1981).

Representative isolates were tested for virulence by intravenous pathogenicity index (IVPI) tests in six-week-old SPF chickens. Furthermore, nucleic acid from the viruses isolated was subjected to nucleotide sequencing in the region of the genome coding for the cleavage site of the haemagglutinin molecule as described by Wood et al. (1994, 1997). The latter tests were performed at the EU Reference Laboratory for Avian influenza and Newcastle disease, Weybridge U.K.

Serology was mainly performed with the haemagglutination inhibition tests using homologous antigen, since turkeys are vaccinated with inactivated multivalent vaccine containing H6 and H9 antigens that interfered with the AGP test.

Histopathology was performed by staining 3µm-thick sections of paraffin embedded tissues with hematoxilin-eosin.

Results

Clinical, post-mortem and histopathological findings

In turkeys reared for meat the severity of the clinical and post mortem disease varied considerably, with mortality ranging from 5% to 97% in depending on the age of the affected birds. The clinical signs were dominated by respiratory distress which started with rales and snicking and then developed into severe dispnoea, associated with swelling of the infraorbital sinuses and conjunctivitis. This condition was always accompanied by complete loss of appetite, febrile condition, ruffled feathers and depression. In some cases, an involvement of the pancreas which appeared haemorrhagic and hardened was observed and histologically, pancreatitis with severe, extensive 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. Intestinal oedema was also present, associated with. fibrinous peritonitis affecting both pancreas and intestine. No other relevant lesion was detected in other districts.

In older birds this clinical situation regressed with recovery in most of the affected birds, while in younger birds, up to 40 days of age, this clinical condition evolved into a more severe respiratory alteration, which in some cases determined air sac rupture, developing in a subcutaneous emphysema, and was associated with mortality rates ranging from 40 to 97%. The most striking post mortem lesion that was present in birds form a large majority of the affected flocks was the presence of a fibrinous clot in the sinuses and trachea, which appeared in most cases to have caused death by suffocation. The trachea and lungs appeared to be congested and in some cases haemorrhagic. Petechial haemorrhages were also present in some cases on the epicardium.

A milder form of the same clinical condition was also observed in turkey breeders which consistently exhibited rales, coughing and swelling of the infraorbital sinuses and a febrile condition associated with loss of appetite. Egg production dropped by 30% to 80% during the acute phase, but partially recovered to subnormal levels within three weeks from the onset of the disease. Egg quality also decreased with misshapen, fragile and whitish eggs being produced during the drop in egg-drop production. Mortality rates ranged from 5 to 20%, while morbidity rates reached 100%. The post mortem findings were of affected respiratory and reproductive tracts with congestion of lung and trachea, sinusitis and conjunctivitis and often the so-called “egg-yolk peritonitis” was observed. A milder condition was seen in affected guinea fowl breeders, with conjunctivitis being a severe and consistent clinical sign.

Only a limited number of broiler and broiler breeder flocks was affected. In broiler breeders, an initial loss of appetite was followed by a drop in egg production of 5-20%. During this phase cyanosis of the combs and wattles could be seen. All the flock appeared to be affected and mortality ranged from 3-8%. Similarly to the turkey breeders, misshapen eggs were also produced in considerable quantity. Pathological findings were restricted to the ovary and oviduct with colliquation of ovarian follicles, associated to catarrhal or fibrinous peritonitis. The only other lesion that appeared to be consistent was the congestion of lung and trachea, which in some cases appeared as pulmonary oedema.

In broiler chickens, H7N1 infections were inapparent in some flocks and in others characterised by anorexia and mild respiratory signs with mortality rates that were generally low in the order of 2-3%. In one case mortality reached 20%. The post mortem lesions were limited to the lungs and tracheas, which appeared congested with associated catarrhal tracheitis.

Outbreaks in commercial layers were similar to those observed in broiler breeders, initial signs were loss of appetite and depression, followed by drops in egg production which ranged from 3 to 10%, in some cases egg production losses reached 30%. Recovery to pre-disease levels was obtained in only a few cases, while egg production in most flocks remained 2-3 points below expected levels. Clinical signs were usually present in about 20% of the birds, but mortality never exceeded 5%. Gross lesions mainly involved the reproductive organs and abdominal cavity: the ovary and oviduct appeared oedematous, and on opening the oviduct it contained a catarrhal exudate and fibrin clots, often it was associated with fibrinous and egg-yolk peritonitis. The lungs and tracheas at times appeared congested.

Guinea fowl broilers exhibited respiratory signs similar to those observed in meat-turkeys accompanied by nervous signs with opisthotonus, torticollis and paralysis of the wings with mortality reaching 30%.

Bacteriology

Routine bacteriology sometimes yielded bacteria such as *E. coli, R. anatipestifer* and *P. multocida*, which were considered as secondary agents.

Virology and serology

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 of the H7N1 subtype. The IVPI test performed on a number of isolates gave a result of 0.0. The deduced amino acid sequence of the region coding for the cleavage site of the haemagglutinin molecule for the early isolates was ...PEIPKGR*GLF...., while for some other strains obtained subsequently it was ...PEVKGR*GLF..., both sequences being typical of low pathogenicity viruses.

Seroconversion was observed in all suspected flocks. Generally speaking, the mean HI titre was higher for turkeys (1:128) than for chickens (1:32).

Discussion

Data collected in the H7N1 1999 Italian epidemic confirm the clinical and pathological findings reported in other LPAI outbreaks (Easterday et al., 1997). In particular, due to the non pathognomonic clinical signs and lesions present in adult birds, the possibility of initially misdiagnosing the clinical condition can lead to a delay in identifying the agent, thus resulting in a considerable spread of infection in a relatively short period of time. Specific virological and serological monitoring programmes should be implemented in order to diagnose infection promptly.

LPAI can be a devastating disease in turkey poults, causing up to 97% mortality in affected flocks. The capability of the virus to spread and infect consistent numbers of flocks apparently virtually simultaneously, may cause great economic losses to the poultry industry, forcing some companies out of business.

Recent scientific evidence (Swayne et al.1998) indicates that LPAI may mutate to HPAI and therefore, LPAI viruses are potentially even more hazardous to the poultry industry. The capacity of the virus to mutate has also occurred in this epidemic, since two different deduced amino acid sequences have been detected. Moreover, the possibility of humans being infected with LPAI (Kurtz et al.,

1996) and HPAI (Claas et al.,1998, Suarez et al. 1998; Subbarao et al. 1998) places these viruses in a different position compared to the one they held when Directive 92/40/EC was drafted. Considering these two points, it would seem advisable that an eradication policy should be implemented in infected areas. However, stamping out of significant numbers of infected flocks is only realistically feasible if there is financial support available, but at the moment this appears to be inapplicable for LPAI. In the framework of current EU legislation the only alternative to a stamping out policy is a control strategy based on the adoption of strict biosecurity measures at a farm level associated to the enforcement of restriction measures and veterinary surveillance in the infected area and the possible use of vaccination. The latter, reduces but does not prevent the virus from replicating (Easterday et al., 1997) and is therefore useless from a biological point of view, nevertheless, it will protect birds form clinical disease and therefore reduce the economic losses. Under current legislation, possible implications of a vaccination policy on intra- or extra-community trade should be taken into account.

A reconsideration of European Union legislation could possibly be a solution to a number of problems which have emerged in the Italian 1999 H7N1 epidemic and in other recent epidemics in which it appeared that LPAI outbreaks caused by H5 and H7 subtypes must be controlled in order to reduce the risk of human infection and avoid heavy economic losses to the poultry industry in Europe .

Acknowledgements

The authors wish to thank D.J. Alexander, R.J. Manvell and J. Banks of the EU Community Reference Laboratory for performing virulence tests and sequencing isolates. The precious technical assistance of Barbara Grossele, Marilena Campisi Lo Schiavo, Barbara Tramontan and Laura Boscarato is gratefully acknowledged

References

ALEXANDER D.J., & D. SPACKMAN (1981): Characterization of influenza A viruses isolated from turkeys in England during March-May (1979) Avian Pathology 10, 281-293

CLAAS C.J., A.D.M. OSTERHAUS, R. BEEK, J. DE JONG, G.F. RIMMELZWAAN, D.A. SENNE, S. KRAUSS, K.F. SHORTRIDGE, R.J. WEBSTER, (1998): Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 351, 472-477

CEC (1992): Council Directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. Official Journal of the European Commission, L167, 1-15

EASTERDAY B.C., V.S. HINSHAW, D.A. HALVORSON (1997): "Influenza" In Diseases of Poultry 10th Edition. Edited by B.W Calnek, Iowa State University Press. Ames, Iowa, USA.

KURTZ J., MANVELL R.J., BANKS J. (1996): Avian influenza virus isolated from a woman with conjunctivitis. Lancet 348, 901-902

SUAREZ, D.L., M.L. PERDUE, N. COX, T. ROWE, C. BENDER, J. HUANG & D.E. SWAYNE (1998): Comparison of highly virulent H5N1 influenza a viruses isolated from humans and chickens from Hong Kong. Journal of Virology, 72, 6678-6688.

SUBBARAO, K., A. KLIMOV, J. KATZ, H. REGNERY, W. LIM, H. HALL, M. PERDUE, D. SWAYNE, C. BENDER, J. HUANG, M. HEMPHILL, T. ROWE, M. SHAW, X. XU, K. KUKUDA & N. COX (1998): Characterization of an avian Influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science, 279, 393-396.

SWAYNE D.E., J.R. BECK, M. GARCIA, M.L. PERDUE, M. BRUGH (1998): Pathogenicity shifts in experimental Avian Influenza virus infections in chickens. Proceedings of the Fourth International Symposium on Avian Influenza, Athens, Georgia pp.171-181. Edited by D.E.Swayne and R.D. Slemons. Georgia Center for Continuing Education, The University of Georgia, Athens Georgia USA.

WOOD G.W., J. BANKS, J.W. MCCAULEY & D.J. ALEXANDER (1994): Deduced amino acid sequences of the haemagglutinin of H5N1 avian influenza virus isolates from an outbreak in turkeys in Norfolk, England. Archives of Virology 134, 185-194

WOOD G.W., J. BANKS, L.H. BROWN, I. STRONG & D.J. ALEXANDER (1997): The nucleotide sequence of the HA1 of the haemagglutinin of an H1 avian influenza isolate from turkeys in Germany provides additional evidence suggesting recent transmission from pigs. Avian Pathology 26, 347-355

EU REPORTS

REPORT OF THE EUROPEAN UNION REFERENCE LABORATORIES FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE 1999

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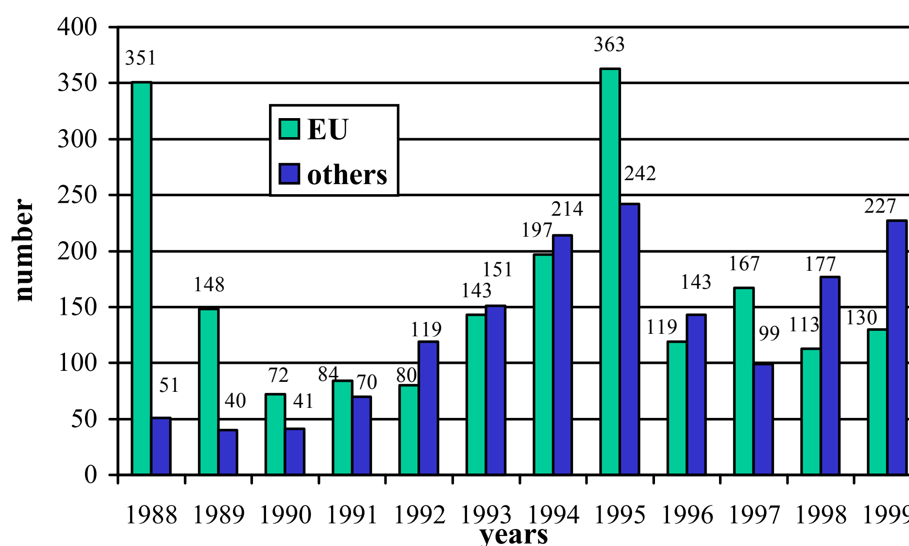
Viruses received by International Reference Laboratory

The numbers of viruses submitted by all countries to the reference laboratory for each of the years 1988 to 1999 are shown in Figure 1. They include viruses submitted under the auspices of the OIE and FAO. The number of viruses submitted in a particular year does not necessarily mean that the viruses were isolated that year and the reference laboratory is often asked to characterise isolates retrospectively.

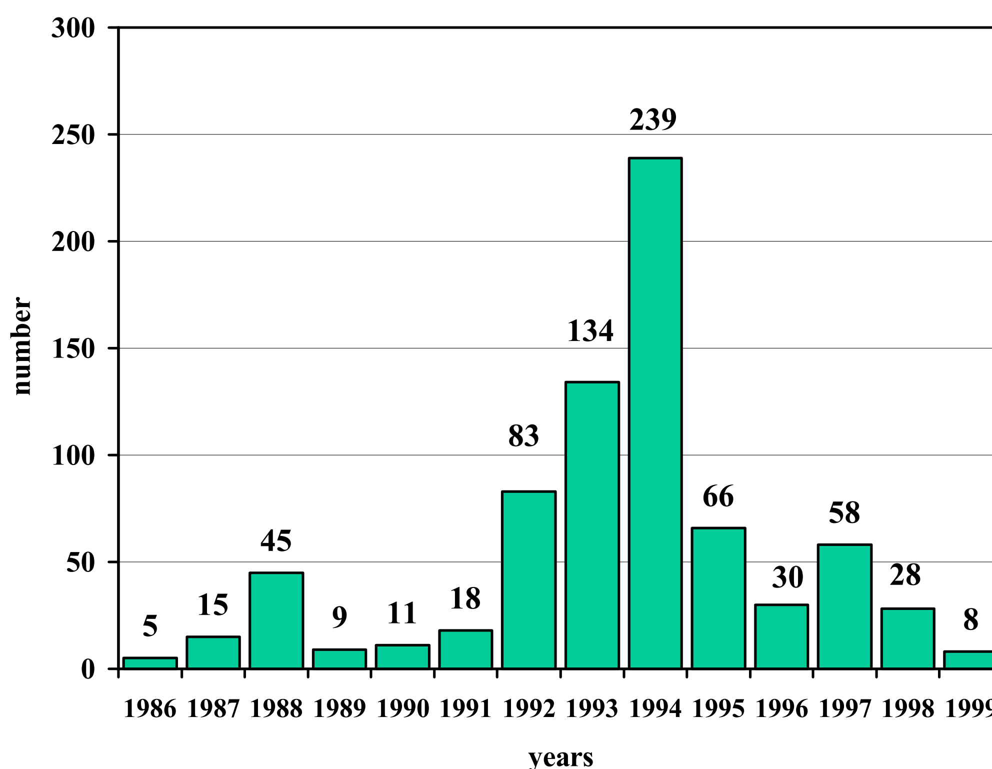
Figure 1. Viruses submitted to the International Reference Laboratory VLA Weybridge during 1988 to 1999 [full year]



The total number of submissions received in 1999, 357, is higher than in recent years. This is unusual because submissions, especially those from the EU usually

reflect the number of outbreaks of Newcastle disease and in 1999 there were only 8 reported in the EU (Figure 2) and equally low numbers of reports elsewhere.

Figure 2. Reported outbreaks of Newcastle disease in EU countries 1986-1999.



Influenza viruses submitted during 1999

Influenza virus submissions are listed in Table 1. Between 01.01.99 and 30.11.99 92 were received from non-EU countries and 80 from EU member states.

Viruses received from EU countries were dominated by the H7N1 viruses of low virulence submitted from Italy [see country report]. Twelve H9N2 viruses were submitted by laboratories in The Netherlands [9] and France [3], but these had originated in countries where viruses of this subtype were prevalent.

The H9N2 viruses submitted from Iran, Pakistan, Saudi Arabia and Korea reflected the widespread disease problems in poultry in Asia and the Middle East as a result of infections with this virus of low pathogenicity for chickens in laboratory infections. Few other influenza viruses were submitted due to outbreaks in the field. The large number of viruses submitted by Taiwan represented a collection built up over a number of years.

The single highly pathogenic influenza virus submitted during the period was isolated from a peregrine falcon in the UAE. This virus was of H7N3 subtype and phylogenetic analysis based on the HA1 gene sequence suggested it was extremely closely related to the viruses responsible the HPAI outbreaks in Pakistan in the mid1990s.

Table 1: Influenza A viruses submitted to the International Reference Laboratory by EU countries during 01.01.99 to 30.11.99.

Country	H1N1	H2N2	H3N8	H5N2	H6N1	H6N2	H7N1	H9N2
Italy							60	
France			1*					3*
Germany		2			1	2		
Spain	1							
Netherlands								9*
Belgium				1				

*viruses did not originate in these countries

Table 2: Influenza A viruses submitted to the International Reference Laboratory by non-EU countries during 01.01.99 to 30.11.99.

Country	H1 N1	H1 N3	H2 N3	H3 N8	H4 N2	H4 N6	H4 N7	H4 N8	H6 N8	H7 N1	H7 N3	H7 N7	H9 N2	H10 N7	H10 N9
S.Africa									1			1			6
UAE											1				
Iran													34		
Pakistan													14		
S.Arabia													5		
Korea													4		
Taiwan	2	2	1	1	1	13	1	1		3				1	

Nucleotide sequencing H5 and H7 viruses.

Following RT-PCR and nucleotide sequencing the amino acid sequence at the cleavage site of the haemagglutinin precursor protein of each H5 and H7 virus submitted was deduced. In keeping with the *in vivo* virulence tests the only virus that had multiple basic amino acids at the cleavage site of the HA0 protein was the H7N3 virus from the peregrine falcon in the UAE (Table 3). This virus had an IVPI of 1.46, which although higher than the value of 1.2, the EU definition of HPAI, is unusually low for HPAI viruses. Two different cleavage sites were seen with the Italian H7N1 viruses differing by a isoleucine or valine at position –5 from the cleavage position (Table 3).

Table 3. Deduced amino acid sequences at the HA0 cleavage site of H5 and H7 viruses submitted to the International Reference Laboratory during 01.01.99 to 30.11.99.

Virus	IVPI	amino acid sequence at the HN0 cleavage site
poultry/Italy/99 (H7N1) [x 51]	0.00	PEIPKGR↓GLF
poultry/Italy/99 (H7N1) [x 9]	0.00	PEVPKGR↓GLF
chicken/Belg./99 (H5N2)	0.00	PQRETR↓GLF
ostrich/S.Africa/99 (H7N1)	0.00	PEIPKGR↓GLF
teal/Taiwan/98 (H7N1) [x 3]	0.00	PEIPKGR↓GLF
per.falcon/UAE/98 (H7N3)	1.46	PETP**KRRKR**↓GLF

Paramyxoviruses received during 01.01.99 to 30.11.99

The low number of paramyxoviruses received from EU countries in 1999 (Table 4) was consistent with the low Newcastle disease situations reported. All viruses typed as APMV-1, but distinct from the pigeon variant PPMV-1, were of low virulence for chickens and most were isolations of live vaccine viruses.

Table 4: European Union countries submitting APMV viruses during 01.01.99 to 30.11.99.

Country*	APMV-1	PPMV-1	APMV-2
Great Britain	1	16	
Ireland	3		
Germany	1	5	
France	1		
Italy	2	2	7
Total	**8**	**23**	**7**

**Not all viruses received had been necessarily isolated in 1999 and numbers may include viruses isolated in countries other than the submitting country.*

In 1999 totals of 94 viruses typed as APMV-1 [4 were PPMV-1] and 5 viruses type as APMV-2 (Table 5), were received from 10 countries from outside the EU representing a wide cross section from different continents and geographical areas. In addition a further 32 viruses were submitted for identification that proved not to be APMVs.

Table 5: Other countries submitting APMV viruses during 01.01.99 to 30.11.99*.

Country	APMV-1	PPMV-1	APMV-2	not APMV
South Africa	13	1	1	6
Saudi Arabia	4		4	
UAE	36	3		25
India	4			1
Philippines	6			
Canada	5			
Singapore	3			
Ethiopia	1			
Switzerland	1			
New Zealand	17			
Total	**90**	**4**	**5**	**32**

**Not all viruses received had been isolated in 1999.*

Antigenic grouping of Newcastle disease virus [APMV-1] isolates.
All APMV-1 viruses received during 1999 were characterised by their ability to cause binding of a panel of 28 monoclonal antibodies [mAbs] to cell cultures infected with the viruses. For convenience and the need to summarise, the results are shown in mAb groups (Alexander et al, 1997) in Tables 6-7. Generally, viruses in the same group show identical binding patterns for all 28 mAbs used. However, some isolates in the same group may vary by their ability to react with one or two mAbs.

All viruses placed in the same group tended to share the same biological properties and for convenience the groups have been separated in Tables 5-6 on the basis of virulence for chickens.

Acknowledgements
The nucleotide sequencing of the influenza viruses was done by Jill Banks and Emma Speidel.

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

Table 6. Antigenic grouping of APMV-1 [Newcastle disease virus] isolates from EU countries submitted during 01.01.99 to 30.11.99

Country	Number of viruses in monoclonal antibody binding group			
	velogenic	*lentogenic*		
	P	**G**	**E**	**?[a]**
Great Britain	16			1
Germany	5			1
France		1		
Republic of Ireland		1	2	
Italy	2			2

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

Table 7. Antigenic grouping of APMV-1 [Newcastle disease virus] isolates from non-EU countries submitted during 01.01.99 to 30.11.99.

Country	Number of isolates in monoclonal antibody binding group									
	velogenic						*lentogenic*			
	P	**B**	**C1**	**J**	**?[a]**	**D**	**F**	**E**	**G/Q**	**?[a]**
South Africa	1		12		1					
Saudi Arabia					4					
UAE	3	8	14	9	4					
India			1	1		1		1		
Philippines			3					3		
New Zealand									17	
Canada						4	1			
Singapore										3
Ethiopia									1	
Switzerland								1		

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

ANIMAL HEALTH REQUIREMENTS RELATED TO TRADE IN RATITES AND RATITE MEAT (Doc.XXIV/2950/99)

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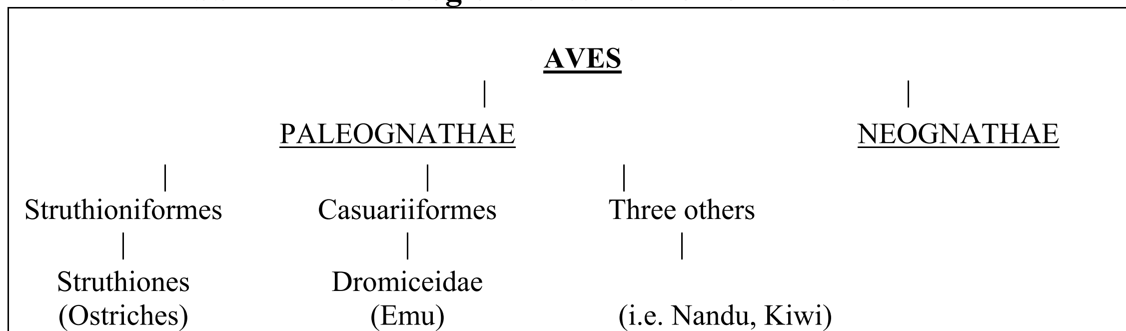
I. Introduction

Ratites (ostriches, emu and others) can be defined on the basis of various aspects. Zoologically they are Paleognathae and belong to a chain different from that of most of the other birds (Neognathae). As for their management they can be kept like domesticated birds or can be regarded as wild animals. Concerning their susceptibility to diseases and the spread of diseases this seems to be comparable to that of other birds.

Abb. 1: Zoological classification of ratites



Veterinary Community legislation reflects the difficulties in defining ratites: They are considered as poultry but they also fall under legislation for farmed game and under that for wild game.

II. Ratites in community animal and public health legislation

II.1 Live ratites and their hatching eggs

II.1.A Ratites as poultry

Ratites fall under the definition of poultry according to Article 2 (1) of Council Directive 90/539/EEC on animal health conditions governing intra-Community trade in and imports from third countries of poultry and hatching eggs.

State of harmonisation of imports:

Principal list of third countries: Decision 95/233/EC
Harmonised list of third countries: Decision 96/483/EC (excludes ratites and hatching eggs)
Certificate: Decision 96/482/EC (excludes ratites/hatching eggs)
For the latter two drafts are being prepared.

II.I.B Ratites other than poultry
Trade in birds other than those referred to in Directive 90/539/EEC (see above) must meet the requirements laid down in Article 7 of Council Directive 92/65/EEC. With regard to ratites, Directive 90/539/EEC is applicable only for those reared or kept in captivity for breeding, the production of meat or eggs for consumption, or for re-stocking supplies of game. It is, for example, not applicable for birds for exhibitions, shows or contests. These ratites fall under Directive 92/65/EEC.

For these birds no lists of third countries or certificates have been laid down on Community level so far.

II.2 Fresh ratite meat

II.2.A Ratite meat as poultry meat
Animal health requirements for fresh poultry meat including fresh ratite meat are laid down in Council Directive 91/494/EC on animal health conditions governing intra-Community trade in and imports from third countries of fresh poultry meat.

By definition fresh ratite meat is fresh poultry meat, as Article 2 of Council Directive 91/494/EC refers to the definitions laid down in Directive 90/539/EEC.

Meat of ratites is not included in the definition of poultry meat given in Article 2 (1) of Council Directive 71/118/EEC on health problems affecting trade in fresh poultry meat.

State of harmonisation of imports:

Principal list of third countries: Decision 94/85/EC
Harmonised list of third countries: Decision 94/984/EC
Certificate (animal health): Decision 94/984/EC (excludes ratite meat)
List of establishments: Decision 97/4/EC

A certificate for ratite meat has been prepared and is to be agreed shortly.

II.2.B Ratite meat as farmed game meat

1. Animal and public health requirements for farmed game meat including ratite meat are laid down in Council Directive 91/495/EEC concerning public health and animal health problems affecting the production and placing on the market of rabbit meat and farmed game meat.

   'Farmed game' is defined in Article 2 (3) of Council Directive 91/495/EC as - in this context - birds, which are not considered as domestic and not referred to in Article 2 of Council Directive 71/118/EC, but which are farmed as domestic animals. Farmed ratites are therefore included.

   By the definition given in Article 2 (2) of Council Directive 91/495/EC 'farmed game meat' includes meat from all wild birds, including the species referred to in Article 2 (1) of Directive 90/539/EEC, bred, reared and slaughtered in captivity which are fit for human consumption. Thus ratite meat is included.

2. Animal and public health requirements for farmed game meat are also laid down in Council Directive 92/118/EC laying down animal and public health requirements governing trade in and imports into the Community of products not subject to the said requirements laid down in specific Community rules referred to in Annex A (1), to Directive 89/662/EC and, as regards pathogens, to Directive 90/425/EC.

State of harmonisation of imports of farmed game meat:

Principal list of third countries:	Decision 94/85/EC
Harmonised list of third countries:	Decision 97/217/EC (excludes ratites)
Certificate:	Decision 97/219/EC (excludes ratites)
List of establishments:	Decision 97/467/EC[1]

II.2.C Ratite meat as wild game meat

Ratite meat is also wild game according to Council Directive 92/45/EEC on public health and animal health problems relating to the killing of wild game and the placing on the market of wild-game meat. In Article 2 (1) a) wild game is defined - in the context of birds – as wild birds which are not covered by Article 2 of Council Directive 91/495/EEC concerning public health and animal health problems affecting the production and placing on the market of rabbit meat and farmed game meat. Wild ratites not covered by Article 2 of Directive 91/495/EEC are therefore those, which are not farmed as domestic animals

[1] According to Article 1 farmed game meat imports may be authorised by the Member States from the establishments of third countries listed in the Annex. Article 2a of this Decision, however, allows inclined Member States to authorise establishments for import of ratite meat up to 1 October 2000.

State of harmonisation of imports:

Principal list of third countries:	Decision 94/86/EC
Harmonised list of third countries:	Decision 97/217/EC (excludes ratites)
Certificate:	Decision 97/218/EC (excludes ratites)
List of establishments:	Decision 97/468/EC[2]

II.3 Ratite meat products

For ratite meat products (farmed game and wild game meat) the provisions of Council Directive 77/99/EEC have to be applied.

State of harmonisation of imports:

Harmonised list of third countries:	Decision 97/222/EC (farmed and wild game meat)
Certificate (animal health):	Decision 97/221/EC (farmed and wild game meat)
Certificate (public health):	Decision 97/41/EC (farmed and wild game, poultry)
List of establishments:	Decision 97/569/EC

II.4 Ratite meat preparations

For ratite meat products (farmed game and wild game meat) the provisions of Council Directive 94/65/EC laying down the requirements for the production and placing on the market of minced meat and meat preparations are applicable.

State of harmonisation of imports:

Harmonised list of third countries:	Decision 94/984/EC farmed and wild game meat)
Certificate (public health):	Decision 97/29/EC (poultry, farmed and wild game)
List of establishments:	Decision 1999/710/EC

An animal health certificate for ratite meat preparations has not been laid down thus import rules for these products are not yet harmonised.

III. Animal health requirements related to trade

Despite the various legislative acts applicable for ratites and their products Community animal health legislation requires in general the same guarantees as

[2] In this Decision a number of establishments from which Member States are allowed to authorise imports of wild feathered game meat are listed.

for “normal” poultry. This includes in particular the measures foreseen for the control of avian influenza and Newcastle disease, such as compulsory notification of the disease, movement controls, killing of infected animals etc. However, due to the peculiarities in the management of ratites, in the trade of their products, their economic value and, as scientific research seems to indicate, in their diseases, animal health requirements need to be adapted accordingly.

Recent changes in Directives 90/539/EEC and 91/494/EEC now allow for such derogation from the general requirements. For this purpose, rules need to be drawn up offering animal health guarantees at least equivalent to those offered by the rules governing intra-community trade. Such special rules could for example include the points listed below.

1. If meat shall be imported from third countries free from AI and ND, the same requirements as for other poultry could apply.
2. If the meat shall be imported from third countries not free from ND the special conditions could include:
   - 2.1 Imports only of de-boned and de-skinned meat
   - 2.2 No outbreaks in the holding of origin and in a defined radius around the holding for a certain time
   - 2.3 Approved testing regime
     - in countries with ND vaccination
     - in countries without ND vaccination
   - 2.4 Approved testing regime in case of vaccination.

Commission Report

AVIAN INFLUENZA AND NEWCASTLE DISEASE IN THE EUROPEAN COMMUNITY: LEGISLATIVE ASPECTS (Doc.XXIV/2913/99)

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DISEASE NOTIFICATION AND SITUATION

The outbreaks of avian influenza and Newcastle disease reported by the Member States in the years 1994 – 1999 are shown in the following tables 1 to 4.

Avian influenza

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

COUNTRY	1994	1995	1996	1997	1998	1999
Austria	0	0	0	0	0	0
Belgium	1	0	0	0	0	0
Denmark	0	0	0	0	0	0
Finland	0	0	0	0	0	0
France	0	0	0	0	0	0
Germany	0	0	0	0	0	0
Greece	0	0	0	0	0	0
Ireland	0	0	0	0	0	0
Italy	0	0	0	7	1	0
Luxembourg	0	0	0	0	0	0
Netherlands	0	0	0	0	0	0
Portugal	0	0	0	0	0	0
Spain	0	0	0	0	0	0
Sweden	0	0	0	0	0	0
United Kingdom	0	0	0	0	0	0
TOTAL	**0**	**0**	**0**	**7**	**1**	**0**

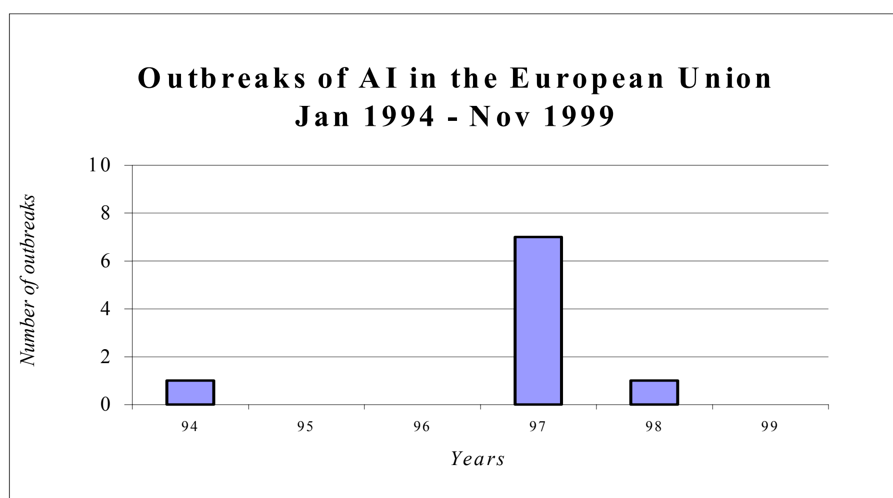

Figure 1: Graphic representation on avian influenza outbreaks 1994-1999

No outbreak of avian Influenza was reported in 1999 in the European Union.

Newcastle disease

Table 2: Number of outbreaks of Newcastle disease reported by the Member States according to the ADNS system

COUNTRY	1994	1995	1996	1997	1998	1999
Austria	0	0	4	2	6	4
Belgium	1	11	7	2	14	0
Denmark	0	14	4	0	2	0
Finland	0	0	2	0	0	0
France	0	0	0	1	1	0
Germany	173	28	2	0	0	0
Greece	0	0	0	0	0	0
Ireland	0	0	0	1	0	0
Italy	42	2	4	2	2	1
Luxembourg	0	2	0	0	0	0
Netherlands	8	5	2	1	0	1
Portugal	12	2	3	10	0	0
Spain	13	0	0	0	0	0
Sweden	0	1	0	1	0	0
United Kingdom	0	0	2	38	0	0
TOTAL	**236**	**65**	**30**	**58**	**25**	**6**

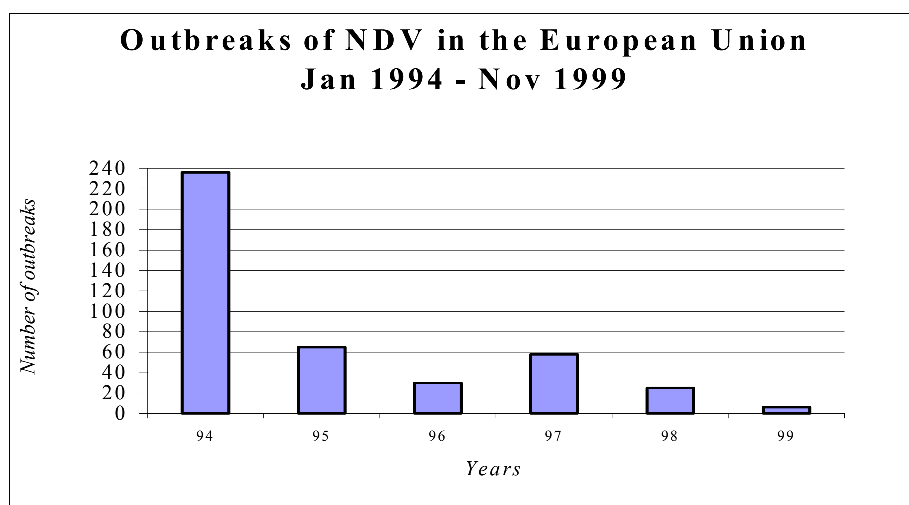

Figure 2: Graphic representation of Newcastle disease outbreaks in the Community

Compared to recent years the number of reported outbreaks has reduced considerably due to stamping-out policy and to vaccination programmes carried out in the different Member States. As most of the outbreaks reported in 1999 occurred in pigeons more emphasis should be laid on the control of these birds and the compulsory vaccination for shows and races.

Control and eradication of avian influenza and Newcastle disease

a) Control measures

The measures adopted to control and eradicate avian influenza and Newcastle disease are based on the strategy of stamping-out infected flocks and controlling the movement of poultry, products originating from poultry, vehicles and any other substances liable to transmit virus.

To ensure such actions in the event of an outbreak, Member States have obligations:

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

Where recourse is taken to emergency vaccination the Member State has to inform the Commission and the other Member States within the framework of the Standing Veterinary Committee. For animals in this designated vaccination area certain movement restrictions have to be applied.

In addition to the obligations listed above, the legislation on the control of avian influenza and Newcastle disease includes requirements for:

- designation of national laboratories and a Community reference laboratory
- control measures to be applied when swill is fed to poultry
- a contingency plan, which each Member State shall present 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 Standing Veterinary Committee reviews the epizootic disease situation within the Community usually once a month. 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) Regionalisation policy

Regionalisation is the application of measures to control and eliminate animal disease from an infected area. It replaces the old policy of applying measures at the borders of the affected country, a policy which is not compatible with the Single Market.

The concept of regionalisation has been accepted as the basis for international policy by OIE and many trading partners. Regionalisation has also been included in the Agreement on the application of Sanitary and phytosanitary (SPS) measures adopted by the World Trade Organisation (WTO). It should restrict trade from the designated region but permit trade from the unaffected part of the country without risk to the Member States.

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

These include:

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

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 avian influenza and Newcastle disease. The level of compensation is normally up to 50% of the Member States‘ costs, which relate to slaughter of animals, cleaning and disinfection and destruction of contaminated materials. Within the framework of the same Decision financial contribution can be made available to cover expenditure on national disease programmes, operation of disease reference laboratories and strengthening veterinary infrastructures.

In 1999 the Commission has adopted two decisions concerning Community financial assistance related to the eradication of Newcastle disease:

Decision 1999/548/EC for Portugal
Decision 1999/578/EC for Denmark

Newcastle disease in Australia

Since 1932 no virulent ND strain has been discovered in Australia. Since 1966 various low virulent strains were identified. In September 1998, the first outbreak occurred west of Sydney. Two more farms were subsequently infected. After successful eradication measures were applied on these farms the next outbreaks occurred in April 1999 in the intensive broiler producing area of the Mangrove Mountain region. Finally 10 farms were detected as infected, killed and destroyed. As a precautionary measure all birds in the 32 commercial poultry farms as well as some non-commercial flocks were stamped out within the infected zone of 3-km radius. In July 1999 the Commission took the decision 1999/549/EC concerning certain protective measures related to Newcastle disease in Australia by banning all trade of live poultry and poultry meat from all the State of New South Wales until the 1 of December 1999. In August 1999 one

outbreak occurred in a multi-age layer flock only 3 km away from the initial outbreak in 1998. From a nearby farm virulent Newcastle disease virus was retrospectively found in samples taken at the time of depopulation for processing under official permit in September 1999. The birds had not shown any clinical signs. Studies of the Australian Health Laboratory indicate that the virus in the last outbreaks is related to the virus, which was responsible for the previous cases. The mutation from an endemic low virulent virus is suspected.

Recently the Commission took the decision to reduce the restricted area as all outbreaks have occurred in a well defined region, but to prolong the ban till the 1 May of 2000 while the results of a planned serological survey covering the whole Sydney Basin area are awaited.

DISCUSSION OF THE FOLLOWING TOPICS:

- Crimean Congo haemorrhagic fever
- proposed EU and OIE new definitions of ND again
- H9N2 influenza virus infections
- emergence of virulent ND virus in Australia
- APMV-1 in pigeons
- comparative test for 2000
- The functions and duties of Reference Laboratories
- Definition of avian influenza

SEROSURVEY FOR ANTIBODIES AGAINST CRIMEAN CONGO HAEMORRHAGIC FEVER VIRUS IN THREE PROVINCES OF NORTH-EASTERN ITALY

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Introduction

Crimean–Congo Haemorrhagic fever (CCHF), is a tick-borne zoonosis, caused by a virus belonging to the *Nairovirus* genus of the *Bunyaviridae* family, that causes in humans a haemorrhagic fever with fatality rates of 30% (Swanepoel, 1995). The disease is widespread in Africa, Asia and Eastern Europe, although, recently evidence of the presence of the virus has been also reported in France, Portugal and former Yugoslavia. The distribution of the virus seems however, to follow the geographical distribution of the *Hyalomma marginatum* tick.

CCHF has recently become a concern to European Union Member States for the importation of live ostriches from endemic countries, since human outbreaks associated with ostrich husbandry practices have been recently reported (Capua, 1998).

The life cycle of the virus includes small mammals and ground frequenting birds, which host the immature stages of the ticks, and cattle and sheep, which serve as hosts of the adult *Hyalomma* ticks. Farm animals and ostriches (Swanepoel 1995, Swanepoel *et al*. 1997) undergo a transient viraemia after being bitten by adult ticks, while human beings develop a severe clinical disease (Swanepoel 1994).

The vector appears to be widely distributed in Italy, and in addition to this, consistent numbers of farm animals are imported into north-eastern Italy from eastern European countries. Since farm animals are the main host of the adult *Hyalomma* ticks, they can be considered an indicator of the presence or absence of infection in a given territory. The aim of this study was to establish whether or not Crimean–Congo Haemorrhagic Fever Virus (CCHFV) was present in the cattle and sheep populations of three provinces of north-eastern Italy.

Materials and methods

A total of 2924 serum samples collected between 1998 and 1999 in the provinces of Belluno, Padova and Vicenza were processed for the detection of antibodies to CCHFV. The blood samples originated from the brucellosis and infectious

bovine rhinotracheitis (IBR) eradication campaigns and were collected from herds or flocks which had been reared in the open for the previous months. The distribution of samples per province and species is reported in Table 1. A greater number of samples originating from the province of Belluno was selected, due to the fact that this province is particularly infested with ticks, and is, in fact, under WHO surveillance for Tick–Borne Encephalitis. The samples were processed by means of a c-ELISA developed by Prof. R. Swanepoel, Special Pathogens Unit, National Institute for Virology, Sandringham, South Africa, as recommended by the EUSCAHAW Document XXIV/B3/R11/1999.

Table 1: Origin, number and species of the serum samples processed for the detection of antibodies to CCHFV.

Origin and species of samples	Belluno	Padova	Vicenza	Eastern Europe	Total
Sheep-goat	992	392	711		2095
cattle	754			75	829
total	1746	392	711	75	2924

Results and discussion

All samples tested gave negative results. Considering that the samples were collected after the summer grazing season, therefore at least a couple of months after maximum tick infestation, the absence of specific antibodies supports the thesis that CCHFV is not circulating in the arthropod or domestic ruminant populations of the provinces examined.

References

Capua I. (1998) Crimean Congo Haemorrhagic fever in ostriches: a public health risk for countries of the European Union? *Guest Editorial. Avian Pathology 27* 117-120

Swanepoel R (1994) Crimean-Congo haemorrhagic fever. In: J.A.W. Coetzer, G.R. Thomson and R.C. Tustin Eds. Infectious diseases of livestock with special reference to Southern Africa 723-729.

Swanepoel R., (1995). Nairovirus infections. In: Porterfield J.S (ed.) Exotic viral infections 285-293. London Chapman and Hall

Swanepoel, R., Leman P.A., Burt F.J., Jardine J., Verwoerd D.J., Capua I., Bruckner G.K. & Burger W.P. (1998) Experimental infection of ostriches with Crimean Congo Haemorrhagic fever virus. *Epidemiology and Infection 121* 427-432

DEFINITIONS OF NEWCASTLE DISEASE

In May 1999 the Office International des Epizooties adopted a new definition of Newcastle disease:-

> *Newcastle disease is defined is an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence:*
>
> *a) The virus has an intracerebral pathogenicity index (ICPI) 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 residues 113 to 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characterisation of the isolated virus by an ICPI test.*
>
> *In this definition, amino acid residues are numbered from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene, 113–116 corresponds to residues –4 to –1 from the cleavage site.*

In essence this definition is very close to the proposed EU definition contained in document XXIV/B3/AHAW/R01/1998 and preferred by the participants at the 5th Meeting in 1998 pp78-79.

The main difference is the use of "....disease of birds..." rather than "....disease of poultry...". However, the intention of the OIE is to recommend different control measures and trading restrictions for different types of bird. Equally the OIE definition could be adopted by the EU member countries, since it is clear in Article 1 of Directive 92/66/EEC that the control measures in that Directive apply only to a) poultry; b) racing pigeons and other birds kept in captivity.

H9N2 INFLUENZA VIRUS INFECTIONS

Dennis Alexander introduced a brief discussion on H9N2 viruses.

During the latter part of the 1990s, submissions to the OIE, FAO International Reference Laboratories or the EU Community Reference Laboratories for avian influenza at VLA Weybridge as a result of infections of poultry with influenza viruses of H9 subtype had been noticeably common.

Outbreaks, due to H9N2 subtype occurred in the following countries:-

Italy in chickens in 1994 (Paparella et al., 1995) and 1996 (Fioretti et al., 1998); **Germany** in domestic ducks, chickens and turkeys during 1995-97 and in 1998 (Werner, 1996; 1999); I**reland** pheasants in 1997 (Campbell, 1998); **South Africa** in ostriches in 1995 (Alexander, 2000); **USA**, in turkeys in 1995 and 1996 (Halvorson et al., 1998); **Korea** in chickens in 1996 (Mo et al., 1998); **China [incl. Hong Kong]** 1992, 1994, 1997-present (Banks et al, 2000).

More recently, H9N2 viruses have been reported in **Middle Eastern countries** and have been responsible for widespread and serious disease problems in commercial chickens in **Iran** (Agakhan et al., personal communication) and **Pakistan** (Naeem et al., 1999) in 1998-99. Infections of chickens with H9N2 viruses in **Korea** and **China** have also occurred in this period. Viruses of H9 subtype have been isolated from feral birds and domestic poultry regularly over the past 30 years (Alexander, 1982, 1993), but never as the dominant H subtype seen in birds or occurring simultaneously over such a wide geographical area. Whether the recent reports of influenza A viruses of the same [H9] subtype from countries from different parts of the world represents spread among poultry or separate introductions from one or different sources of feral birds is unclear. Although the widespread presence and associated disease of H9N2 viruses in so many countries in recent years should be of concern to the National Laboratories, of equal significance and concern are the isolations of viruses of H9N2 subtype from pigs originating in Southern China and two children with influenza-like symptoms in Hong Kong (Peiris et al., 1999a,b).

References

Alexander, D.J. (1982). Avian influenza - recent developments. *Veterinary Bulletin, 52,* 341-359

Alexander, D.J. (1993). Orthomyxovirus Infections. In Viral Infections of Vertebrates Volume 3: Viral Infections of Birds. Edited by J.B. McFerran & M.S. McNulty Series editor M.C. Horzinek, Elsevier Sci. Pub. Co. : Amsterdam (pp287-316).

Alexander, D.J. (2000). A review of avian influenza in birds. Proceedings of the ESVV Symposium on Animal Influenza Viruses, Gent 1999. *Veterinary Microbiology,* in press.

Banks, J., Speidel, E.C., Harris P.A. & Alexander, D.J. (2000). Phylogenetic analysis of influenza A viruses of H9 haemagglutinin subtype. Avian Pathology

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,* 1-12.

Campbell, G. (1998). Report of the Irish national reference laboratory for 1996 and 1997. *Proceedings of the Joint Fourth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 9-10th December 1997, Brussels.* (p13).

Fioretti, A., Menna, L.F. & Calabria, M. (1998). The epidemiological situation of avian influenza in Italy during 1996-1997. *Proceedings of the Joint Fourth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 9-10th December 1997, Brussels* (pp17-22).

Halvorson, D.A. (1998). Strengths and weaknesses of vaccines as a control tool. *Proceedings of the 4th International Symposium on Avian Influenza, 29th-31st May 1997 Athens, Georgia.* U.S. Animal Health Association (pp223-227).

Halvorson, D.A., Frame, D.D., Friendshuh, A.J., & Shaw, D.P. (1998). Outbreaks of low pathogenicity avian influenza in USA. *Proceedings of the 4th International Symposium on Avian Influenza, 29th-31st May 1997 Athens, Georgia.* U.S. Animal Health Association. (pp36-46).

Mo, I.P., Song, C.S., Kim, K.S., & Rhee, J.C. (1998). An occurrence of non-highly pathogenic avian influenza in Korea. *Proceedings of the 4th International Symposium on Avian Influenza, 29th-31st May 1997, Athens, Georgia. U.S. Animal Health Association* (pp379-383).

Naeem, K., Ameerullah, M., Manvell, R.J. & Alexander, D.J. (1999). Avian influenza A subtype H9N2 in poultry in Pakistan. *Veterinary Record,* in press

Papparella, V., Fioretti, A., & Menna, L.F. (1995). *Proceedings of the Joint Second Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 18th-19th October 1994 Brussels.,* (pp.14-15).

Peiris, M., Yam, W.C., Chan. K.H., Ghose, P. & Shortridge, K.F. (1999a). Influenza A H9N2: Aspects of Laboratory Diagnosis. *Journal of Clinical Microbiology, 37,* 3426-3427.

Peiris, M., Yuen, K.Y., Leung, C.W., Chan, K.H., Ip, P.L.S., Lai, R.W.M., Orr, W.K. & Shortridge, K.F. (1999). Human infection with influenza H9N2. *The Lancet, 354*, 916-917.

Werner, O. (1996). Avian influenza : Current situation in Germany. *Proceedings of the Joint Third Annual Meetings of the National Newcastle Disease and*

Avian Influenza Laboratories of Countries of the European Union, 30th-31st October 1995, Brussels. (p12)

Werner, O. (1999). Avian influenza–situation in Germany 1997/98. *Proceedings of the Joint Fifth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 9-10th November 1998, Vienna*. (pp10-11)

EMERGENCE OF VIRULENT ND VIRUS IN AUSTRALIA

Dennis Alexander drew attention to an important finding from the ND outbreaks occurring in Australia.

Until 1998 Australia had been free of virulent ND virus, since the 1932 outbreak (Albiston and Gorrie, 1942), although it had been recognised since 1966 that viruses similar to those placed in the "asymptomatic enteric" pathotype group (Westbury, 1981; Spradbrow, 1987) were present in wild birds and on occasions spread to commercial poultry flocks. Two outbreaks of virulent ND occurred in 1998 and in 1999 a further series outbreaks were reported. Phylogenetic studies showed the virulent viruses to be extremely closely related to each other and to the endemic virus of low virulence. For the virulent viruses to emerge from the endemic virus of low virulence only two point mutations would have been necessary (Table 1). What is even more conclusive is that intermediate mutants with arginine at position 115 and leucine at 117 were isolated as a result of intensive surveillance of poultry flocks in the area undertaken as a result of the outbreaks (M. Rickard personal communication).

Table 1. Nucleotide/amino acid sequence at F0 cleavage site of viruses of high and low virulence isolated in Australia in 1998

Virus	Virulence	Nucleotide/amino acid sequence at F0 cleavage site
1154/98	low	GGA AGG AGA CAG GGG CGT CTT ^{111}GRRQGR*L^{117}
1238/98	high	GGA AGG AGA CAG **<u>A</u>**GG CGT **<u>T</u>**TT ^{111}GRRQ**R**R***F**^{117}
1249/98	high	GGA AGG AGA CAG **<u>A</u>**GG CGT **<u>T</u>**TT ^{111}GRRQ**R**R***F**^{117}

If mutations to virulence do occur it is not clear whether these take place in feral birds and then pass to poultry or occur once the virus has been introduced in poultry, the lack of virulent isolations from feral birds suggests the latter is the more likely.

The meeting discussed the implications of the possibility that viruses of low virulence could mutate to high virulence and looked forward to further details of the 1999 outbreaks and the viruses of low virulence circulating in Australia.

References

Albiston, H.E. and Gorrie, C.J. (1942). Newcastle disease in Victoria. *Australian Veterinary Journal* **18**, 75-79.

Spradbrow, P.B. (1987). Newcastle disease in Australia. In: *Newcastle disease in poultry: a new food pellet vaccine.* The Australian Centre for international Agricultural Research, Canberra, pp. 40-43.

Westbury, H.A. (1981). Newcastle disease virus in Australia. *Australian Veterinary Journal*, **57,** 292-298.

APMV-1 IN PIGEONS

Two topics were discussed concerning isolations of the virus responsible for the pigeon panzootic [PPMV-1] from pigeons:

- Virulence of isolates

The problem of 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 was discussed. The problem was particularly noted 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; and country reports for Belgium and Italy these proceedings].

- Isolation from pigeons

Several laboratories commented on experiencing greater difficulty in obtaining virus isolation of PPMV-1 viruses from pigeons on first passage in embryonated fowls eggs. Two or more passages had become essential and Great Britain reported greater success of virus isolation on first passage in tissue culture than eggs.

Reference

Meulemans, G., Vanderhallen, H., van den Berg, T.P., Decaesstecker, M. and Boschmans, M. Newcastle disease situation in Belgium. (1999) *Proceedings of the Joint Fifth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 9-10th November 1998, Vienna*. (pp35-36)

COMPARATIVE TESTS FOR 2000

The consensus opinion was that the tests were useful in that they offered a valuable test for the National Laboratories.

In view of the results obtained in 1999 it was decided to repeat antigen identification. There was some suggestion that both antigens and sera could be sent, the Reference Laboratory representatives agreed to look into the feasibility of this. There was also a request by one or two National Laboratories that viruses for sequencing could be included. The Reference Laboratory representatives were dubious about the practicality and desirability of sending live viruses but thought it might be possible to send viruses inactivated in TRIzol.

THE FUNCTIONS AND DUTIES OF REFERENCE LABORATORIES (Doc.XXIV/2912/99)

Dr Westergaard presented the proposed work plan for the Community Reference Laboratories for the approval of the meeting.

Essentially these were the same as in previous years. Representatives of National Laboratories expressed their satisfaction with the level of support and advice given throughout the year.

It was suggested that since an essential part of the work done by members of the CRL was to support National Laboratories and the European Commission by means of information and technical advice during epidemics this should be formally added to the duties.

This was agreed and the following work objectives formally approved by the Meeting.

WORK PROGRAMME AND WORK PLAN FOR THE COMMUNITY REFERENCE LABORATORY FOR AVIAN INFLUENZA, 2000

LEGAL FUNCTIONS AND DUTIES

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

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.
(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:
  (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.
(3) Maintain virus repository and distribute viruses from it and reagents necessary for virus characterisation.
(4) Prepare and distribute antisera, antigens and reagents for the inter-laboratory comparison tests.
(5) Analysis of results submitted by National Laboratories for the inter-laboratory comparison tests.
(6) Conduct work to evaluate reported problem areas in diagnosis.
(7) Support by means of information and technical advice National Avian Influenza Laboratories and the European Commission during epidemics.
(8) Prepare programme and working documents for the Annual Meeting of National Avian Influenza Laboratories to be held in 2000.
(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 that may arise during the given period.

WORK PROGRAMME FOR THE COMMUNITY REFERENCE LABORATORY FOR NEWCASTLE DISEASE, 2000

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).

OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2000

1) Collecting and editing of material for a report covering the annual meeting of National Newcastle Disease Laboratories held in Brussels, November 1999.
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 No L 156, p. 76) as amended by Decision 96/619/EC (OJ No L 276, p. 18). This will, at the request of the European Commission or the submitting National Laboratory or at the discretion of the Reference Laboratory, include:
    a) Determining the intracerebral pathogenicity index (ICPI)
    b) Determining the basic amino acid composition adjacent to the cleavage site of the F0 protein in the virus and phylogenetic analysis
    c) Antigenic grouping of viruses
3) Maintain a virus repository and stocks of reagents necessary for virus characterisation. Distribute viruses held in the repository and limited amounts of reagents to national laboratories on request.
4) Prepare and distribute antisera, antigens and reagents for the inter-laboratory comparison tests.
5) Analysis of results submitted by National Laboratories for the inter-laboratory comparison test.
6) Conduct work to evaluate reported problem areas in diagnosis.
7) Support by means of information and technical advice National Newcastle disease Laboratories and the European Commission during epidemics.
8) Prepare programme and working documents for the Annual Meeting of National Newcastle Disease Laboratories to be held in 2000.
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 that may arise during the given period.

DEFINITION OF AVIAN INFLUENZA

There was considerable discussion of the adequacy of the current definition of avian influenza contained in Directive 92/40. This was stimulated by the large number of outbreaks in poultry in Italy caused by an H7N1 virus of low pathogenicity [i.e. not falling within the current definition]. Plus the current theory that highly pathogenic avian influenza viruses arise from H5 and H7 viruses of low pathogenicity by mutation after they have been introduced into poultry.

The general opinion was that, some form of statutory control should be applied to H5 and H7 viruses of low pathogenicity. However, there was some difference of opinion as to whether all H5 and H7 virus infections should be control with the full measures currently reserved for those of high pathogenicity or some lesser control would be sufficient.

The Commission representatives indicated their intention to put a question on the definition of avian influenza to the EU Scientific Committee on Animal Health and Animal Welfare for their advice.

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