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EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL
Directorate E - Food Safety: plant health, animal health and welfare, international aspects
Director

SANCO

05. 04. 2004

Brussels,
SANCO E3/LT/ise D(2004) 530907

Subject: EC/US Veterinary Agreement - EU status for Newcastle disease (Denmark)

Dear Dr DeHaven,

As announced in the last Joint Management Committee I am pleased to enclose a copy of the report recently received from the Danish competent authorities on the Danish animal health status for Newcastle Disease.

As you will see Denmark, in accordance with the provisions of the relevant international standards (OIE code), is considered free from Newcastle Disease as from 1 March 2003.

I would like to remind to you that for the outbreak of the same disease which occurred in the USA at the end of 2002, the Commission adopted to an expeditious regionalisation, in accordance with the relevant provisions of the EC/US Veterinary Agreement and of the said international standards.

As you know with Council Decision 2003/67/EC adopted on 28.01.03, and subsequent amendments, the Commission succeeded in limiting the import restrictions on live poultry including hatching eggs, fresh poultry meat and poultry products to the goods originating from the States of California, Nevada and Arizona. Finally, in line with the favourable epidemiological evolution of the disease (last outbreak on 31 May 2003) Decision 2003/67/EC expired on 1st December 2003 and trade returned to normal.

Unfortunately, even if the last outbreak in Denmark occurred on 28 August 2002, well before the last one in the USA, the entire Danish territory is still considered by APHIS as affected by Newcastle Disease. Trade restrictions are consequently still in place.

This unbalanced situation clearly underlines a lack of symmetry in the legislative behaviour of our two Parties towards the same issues. It is a serious obstacle to the efforts of the Commission to fully implement the regionalisation provisions of the EC/US

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Veterinary Agreement. This factor has been once more strongly underlined by the Member States' delegations when recently called to express their opinion on the US regionalisation for avian influenza. The key factor which has helped my services on this occasion to overcome, although with considerable difficulties, their legitimate reluctance to regionalise the US has been the hope that the action plan launched recently by Commissioner Byrne during his visit in the US gives the expected results for both Parties.

In line with the abovementioned considerations I would again like to formally request to you an expeditious revision of the Newcastle Disease status of Denmark.

Yours sincerely,

for Alejandro Checchi Lang

Annex: Newcastle Disease Outbreaks in Denmark 2002, Final Report

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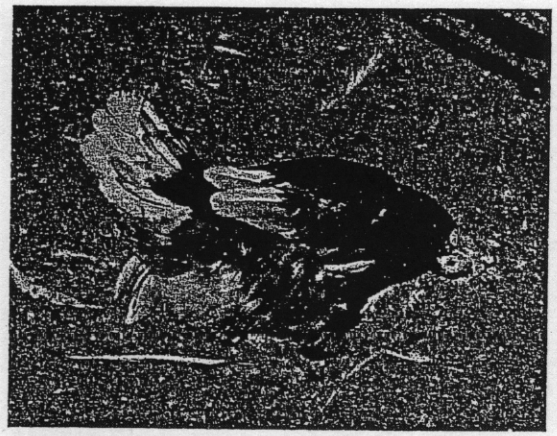
Kingdom of Denmark

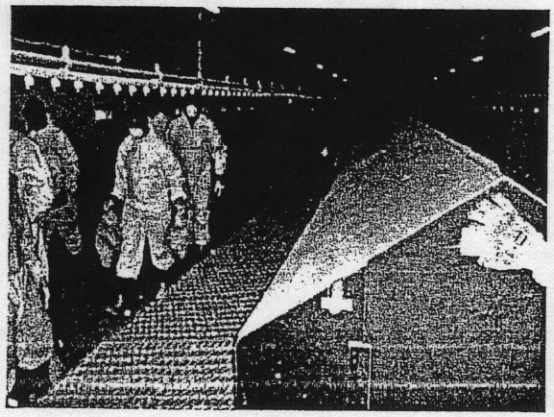
Ministry of Food, Agriculture and Fisheries

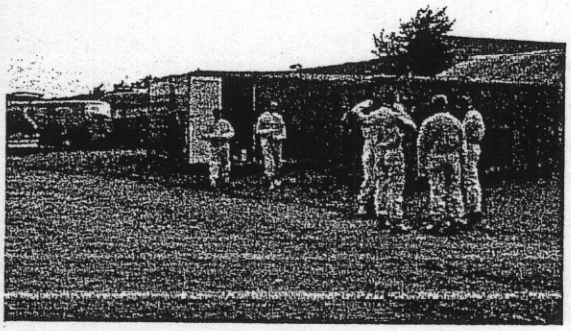
Danish Veterinary and Food Administration

Newcastle Disease Outbreaks in Denmark 2002

Final Report







Danish Veterinary and Food Administration

2003

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Cover Page:

The photographs were taken from Outbreak no. 1 on 27 July 2002, and show pullets with obvious clinical symptoms as well as scenes from the eradication process on two other farms.

Final Report on the Newcastle Disease Outbreaks in Denmark in 2002

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1. Preface

During the outbreaks of Newcastle Disease in Denmark in 2002 three reports were released, with details of the epidemic, epidemiology and progress of the eradication process:

- Report no. 1 of 8 August 2002
- Report no. 2 of 29 August 2002
- Report no. 3 of 5 September 2002

The present report sums up the information and the progress achieved regarding the epidemiology and control of the outbreaks, including the results of the post-epidemic screening, and it presents the conclusion of the epidemic.

For details on each infected flock and other specific information, please refer to the three underlying reports.

Each outbreak is referred to by the serial number, e.g. "outbreak no. 1". This number does not for all cases correspond to the number given when the outbreak was reported to the EU Commission via the Animal Disease Notification System (ADNS). The exact correspondence between the two appears from the table in annex 3 of Report no. 2.

2. Summary

A total of 135 outbreaks were found during the Newcastle Disease (ND) epidemic in Denmark in the summer of 2002. Four primary outbreaks and 131 secondary outbreaks were identified. Most outbreaks were found in the central and southern parts of Jutland, but a single outbreak occurred on the Island of Sejerø in the County of West Zealand. The majority of outbreaks (126) were observed in back-yard flocks. Only 9 commercial flocks were infected. All infected commercial flocks were layers or pullets, whereas no broiler holdings became infected.

The first outbreak was confirmed by virus isolation and ICPI-determination on 26 July 2002 and the last outbreak was confirmed on 28 August 2002. However, eradication of infected flocks, establishment of protection and surveillance zones and restrictions on poultry movements were initiated for precautionary reasons already on the 16 July, ie.10 days before the first virological confirmation was obtained. The preliminary diagnosis was based on clinical signs and strong serological reactions. Positive serology could be used as an indicator of ND infection because Denmark applies a non-vaccinating policy for ND. All poultry on holdings on which the disease was diagnosed have been killed and destroyed.

In the majority of the outbreaks, no obvious clinical symptoms were observed. Among all 135 outbreaks only poultry in 13 outbreaks revealed clinical symptoms such as ataxia, torticollis and egg drop. Seven of these were outbreaks in commercial flocks. The pathological lesions were also limited, as was the tendency to spread by any other means than sale and movement of infected pullets.

As a consequence of the very slow in-vitro growth of the virus involved, the diagnostic methods were primarily based on serology in order to ensure a rapid eradication of the outbreaks. Avian paramyxovirus-1 (APMV-1) was isolated from three flocks of commercial poultry. The two isolates tested both belonged to antigenic group C1 viruses.

Based on the epidemiological investigations, one of the primary outbreaks was linked to all of the131 secondary outbreaks. This was due to the involvement of two poultry dealers, and the infection was spread among back-yard flocks by trade of infected pullets. Thereby, this primary outbreak and the related contacts comprised one large epidemiological cluster of infected flocks.

The epidemiological investigations did not succeed in identifying the source of infection with regard to the four primary outbreaks in commercial flocks. These were all situated less than 2 km from the coastline. The waterfowl populations in the vicinity of these holdings were investigated to locate the potential source of infection, but the results have not been conclusive.

Following preliminary cleaning and disinfection of the affected premises, a serological screening of the more than 600 flocks close to the outbreaks was carried out. A limited prevalence of low serological titres was found in 39 of these flocks, and no virus could be isolated.

During the phases of the eradication process, six regionalisations of Denmark were carried out, in order to successively re-open the export of live poultry and hatching eggs from ND-free regions of Denmark. All restrictions were lifted by 1 January 2003 and Denmark regained its ND free status, according to the Office International des Epizooties (OIE) rules, by 1 March 2003.

3. Background

Newcastle Disease (ND) is classified as an OIE list A disease and is frequently causing high mortality and morbidity in different poultry species. ND is caused by the virus called Avian Para-Myxo-Virus type 1 (APMV-1) with an intracerebral pathogenicity index (ICPI) > 0,7.

Denmark applies a non-vaccination policy against ND according to Council Directive 90/539/EEC, Article 12, established by Commission Decision 91/552/EEC. However, pigeons are compulsory vaccinated against ND in Denmark when they participate in races or exhibitions. The non-vaccination policy implies that additional guaranties may be applied when importing hatching eggs, live poultry and poultry meat. Danish breeding flocks are yearly serologically tested for ND according to Council Directive 90/539/EEC.

Denmark has previously experienced outbreaks of ND in 1995, 1996 and 1998. The outbreak in 1995 involved 14 back-yard flocks. In 1996 one commercial herd, 2 back-yard flocks and 1 flock of game birds were affected. The 1998 outbreak involved 2 commercial flocks of turkeys. In 2000, 2001 and 2002 APMV-1 was isolated from wild living birds.

4. Epidemiology

4.1. Analysis of the epidemic

The 135 confirmed outbreaks occurred in 8 counties in Denmark in July-August of 2002. The first suspicion arose on 13 July 2002 and the last flock was stamped out on 29 August 2002 as shown in Table 1.

Table 1. Outbreaks of Newcastle Disease in Denmark in 2002 by county

County[1]	No. of outbreaks	Suspicion of first outbreak	Date of last occurence[2]
North Jutland	4	18.07.02	27.07.02
Ringkoebing	20	19.07.02	29.07.02
Viborg	2	19.07.02	25.07.02
Aarhus	1	22.07.02	26.07.02
Vejle	1	13.07.02	19.07.02
Ribe	6	20.07.02	27.08.02
Western Zealand	1	16.08.02	21.08.02
Southern Jutland	100	19.07.02	29.08.02

1. The county territory does not necessarily coincide with the Regional Veterinary and Food Authority territory. A map of Denmark showing the affected counties are in Figure 1.
2. Date on which the last stamping-out took place

All but one outbreak were located in Jutland south of Limfjorden, as illustrated in Figure 1. The epidemic was characterised by a relatively late confirmation of the first outbreaks due to difficulties in isolation of the virus. Most of the secondary outbreaks were diagnosed based on serology and contact through trade of birds from one of the primary outbreaks. The spread of the infection was primarily due to the involvement of two poultry dealers, who traded infected pullets to a large number of back-yard flocks. This caused two clusters of outbreaks in back-yard flocks as illustrated in Figure 2.

According to Council Directive 92/66/EEC, the definition of a primary outbreak is isolation of ND virus with an ICPI of 0.7 or above, but during outbreak clinical symptoms or post mortem lesions can be applied to identify secondary outbreaks. As Denmark applies a non- vaccinating strategy for ND, serology was applied to identify secondary outbreaks. From the time of the first positive serological tests on 15 July 2002, and for precautionary reasons, the Danish Veterinary Food Administration (DVFA) based the stamping out policy and establishment of protection and surveillance zones on:

1) Clinical symptoms and positive serology in commercial flocks, or
2) Positive serology and contact (to confirmed or suspected infected poultry), or
3) Repeated positive serology with a change in pattern of titres in paired flock samples.

These criteria were adopted in order to ensure a uniform way to reject or confirm a suspect case. Positive serology was used as an indicator of ND infection. When the first outbreak was officially confirmed on 26 July 2002 by the detection of APMV-1 with an ICPI> 0.7, the described criteria for declaring an outbreak and imposing eradication measures on the holding were maintained.

The first suspicion on 13 July 2002 was in a battery laying hen flock on a farm in Vejle County, and was raised due to clinical symptoms such as increased mortality, paralysis and egg drop. However movement restrictions were immediately imposed at the farm, and following the serological evidence of ND protection- and surveillance zones were established and the flock was stamped out on 19 July 2002. Thus the Danish authorities took precautionary measures and started control measures already before the diagnosis was confirmed virologically.

The second suspicion was raised on 18 July 2002 in a breeding flock in North Jutland, and was also due to clinical symptoms, mainly lameness. As this was the second suspicion with clinical symptoms and furthermore appeared to have many contact flocks, restrictions were imposed on the farm and zones were applied as if the suspicion was confirmed. Epidemiological investigations and sampling in contact flocks was initiated in spite of absence of a virological diagnosis. The second suspicion was supported by positive serology on 19 July 2002. Based on this and the clinical symptoms the pullets were stamped out on 27 July 2002. This was assigned as **outbreak no. 1**, as this outbreak was related to the first isolation of virus, isolated from pullets delivered from this farm to one of the contact farms (outbreak no. 2).

Outbreak no.1 played a major role in the epidemic, as the flock had sold pullets to 6 contact flocks before suspicion was raised and before movement restrictions were imposed on the farm. These contacts consisted of three commercial flocks, two dealer flocks and one back-yard flock. The first virological confirmation on 26 July 2002 (ICPI 1.75) was made in one of the commercial contact flocks (outbreak no. 2). This virologically positive contact flock had received pullets from outbreak no. 1 on 15 June 2002. Due to the epidemiological investigations it was discovered, that these pullets had been affected at the time of delivery and had spread the infection to other pullets residing in the receiving farm.

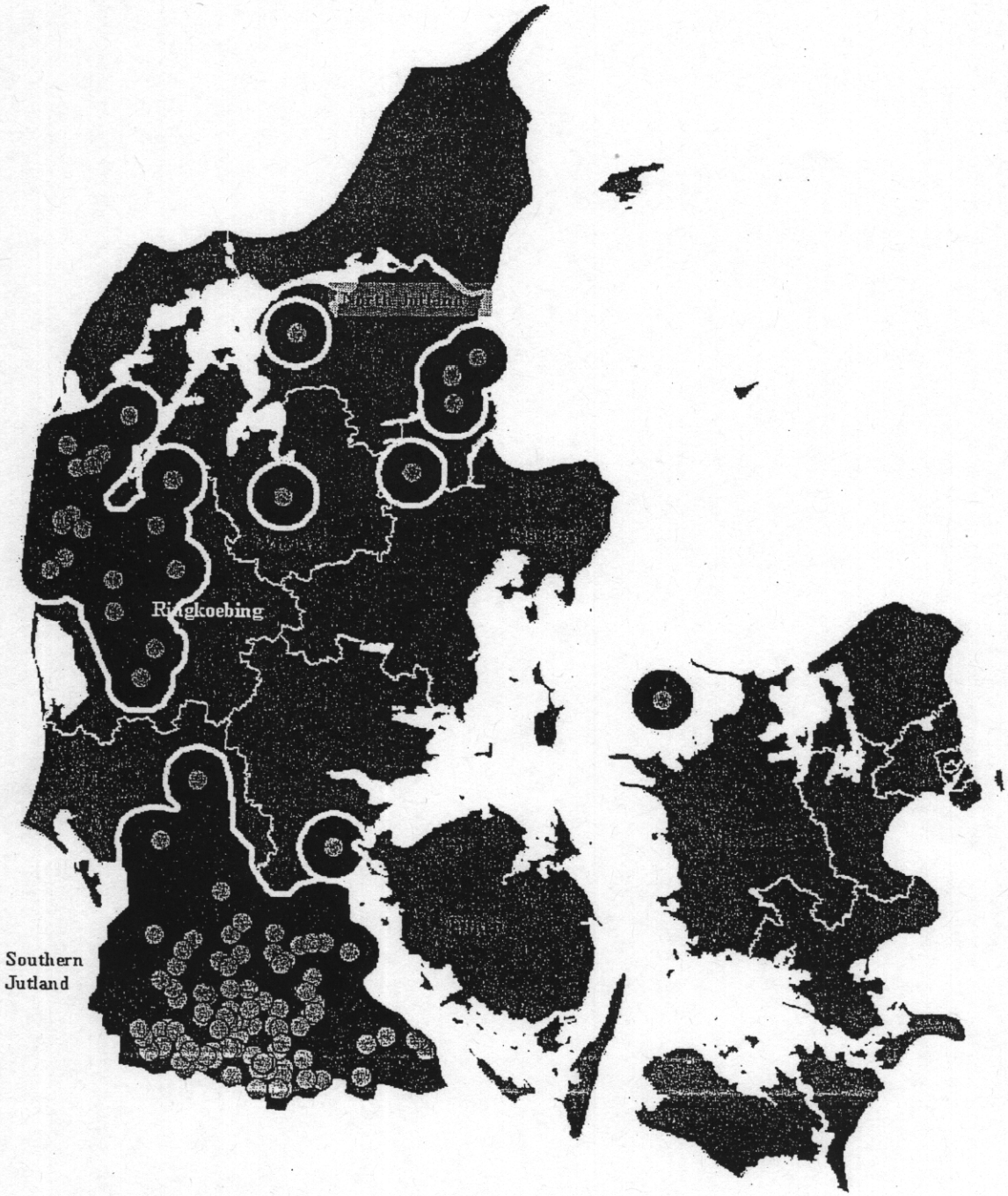

Figure 1. Outbreaks of Newcastle Disease in Denmark 2002.

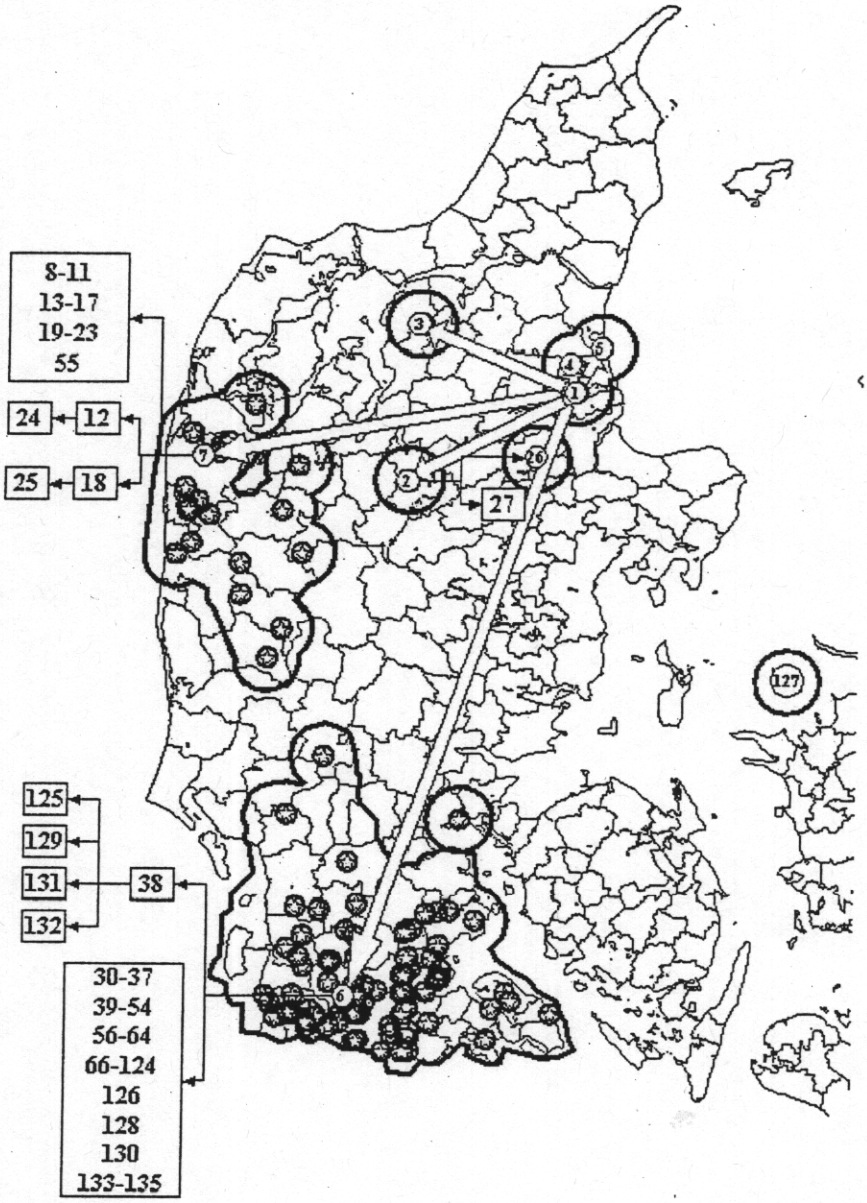

Figure 2. Epidemiological links in the Newcastle Disease epidemic in Denmark in 2002

The two dealers had received pullets on 28 May 2002 and 13 June 2002, respectively and had resold the pullets to a large numbers of back-yard flocks. Therefore outbreak no. 1 ended up being linked to a total of 131 secondary outbreaks, as shown in the cluster link map in Figure 2.

The numbers of poultry involved in the epidemic were relatively low (175.455 birds), as the large majority of outbreaks (91.9%) involved back-yard flocks, as shown in Table 2. A total of 9

commercial flocks were involved in the outbreak of which 2 were breeder flocks, 5 layer flocks and 2 dealer flocks.

These dealers bought poultry at regular intervals, reared them for a short period on their own farm and resold them to back-yard flocks, sometimes selling at weekly fairs or markets. This resulted in the spread of infected pullets to several back-yard flocks primarily in Ringkoebing and Southern Jutland Counties. Tracing of these back-yard flocks was complicated, as one of the dealers did not keep the compulsory records of sales. A large effort had to be made to trace these contact flocks by using phone number records from the dealer, in addition to advertisements in local mass media in order to contact the back-yard flock keepers.

Table 2. Outbreaks of Newcastle Disease in Denmark in 2002 per category of holding

Nature of holding	Numbers of holdings	Percent of total	Numbers of birds
Commercial flocks[1]	7	6.6	157.334
Back-yard flocks	126	91.9	5.958
Commercial dealers	2	1.5	12.163
Total	135	100	175.455

1. Defined as a flock of 1000 birds or more

Figure 3 and Figure 4 illustrate the temporal progress of the outbreaks in commercial and back-yard flocks respectively. The figures show that the outbreaks peaked in late July and early August, mainly due to the intensive investigation of contact flocks with relation to the two involved poultry dealers, i.e. the figures do not describe an epidemic curve as such, but rather the time used for trace-back and detection of infection.

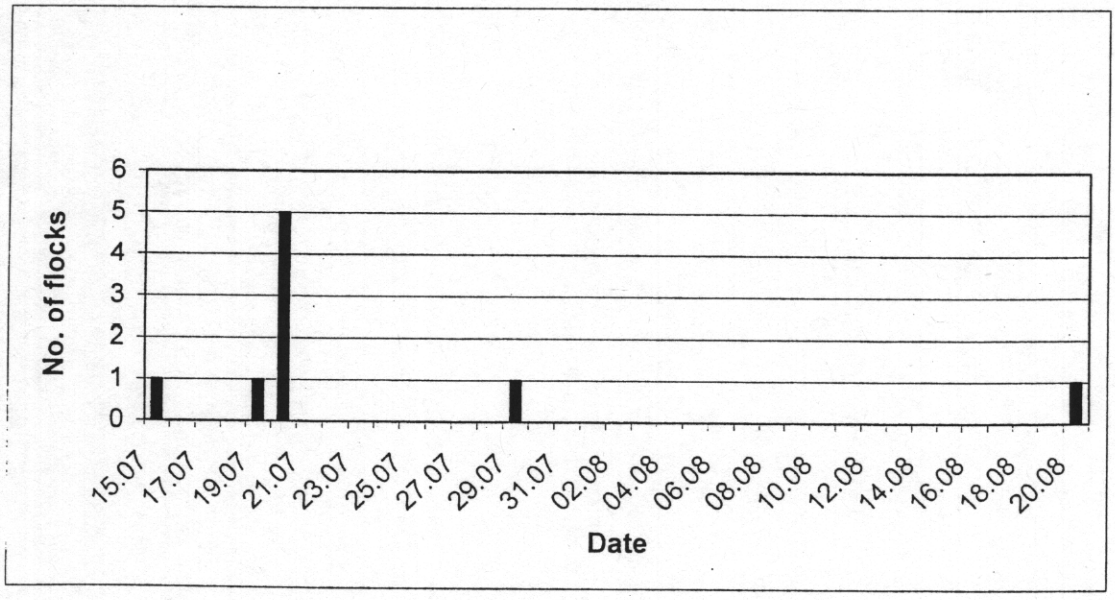

Figure 3. Number of ND-outbreaks in commercial flocks by date of serological result. The four isolated incidences are the four primary outbreaks, while the five serological confirmations on 20 July are the three commercial and the two dealer flocks, which were secondarily infected by prior introduction of pullets from outbreak no. 1 (identified on 19 July).

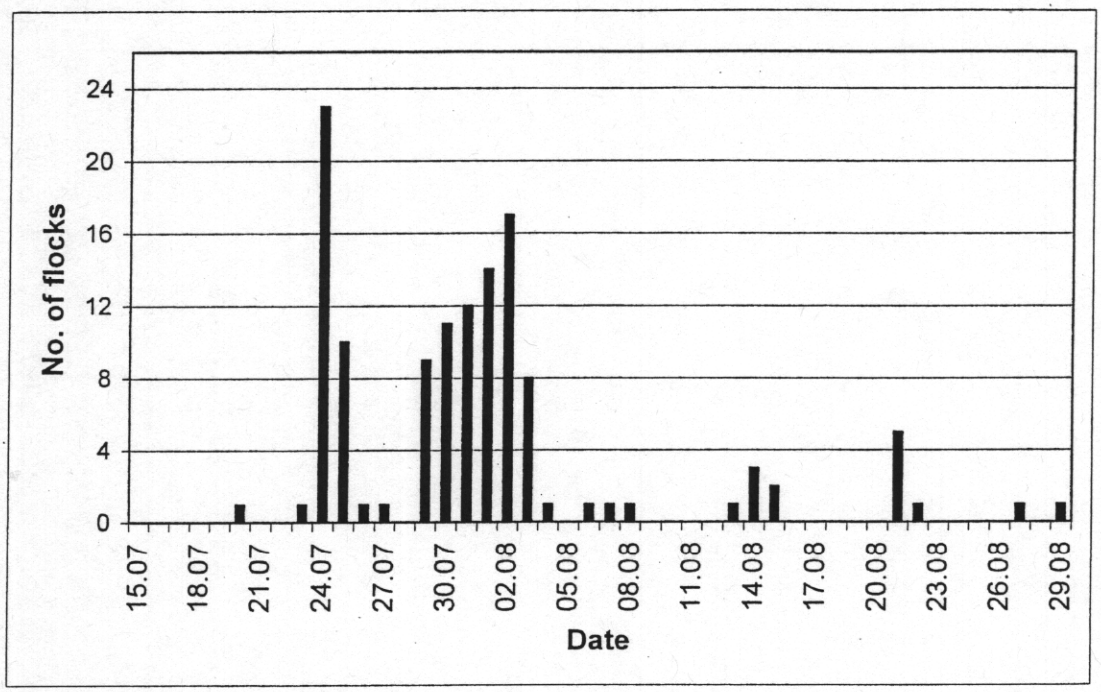

Figure 4. Number of ND-outbreaks in back-yard flocks by date of serological result. All these outbreaks were caused by purchase and transport of infected birds directly or indirectly associated with outbreak no. 1 through two dealer herds (outbreaks no. 6 and 7).

4.2. Epidemiological investigations in commercial flocks

Table 3 shows the characteristics of the nine outbreaks in commercial flocks.

On 13 July 2002 restrictions were imposed on the first suspected flock (outbreak no. 28, situated in Vejle County), by the Regional Veterinary Centre (RVC) because of clinical suspicion of ND. Sampling for virology and serology was conducted on13 July 2002. Two days later the suspicion of ND was supported by the serological results, which revealed high titres (range: 16-4096) indicating an active infection. Furthermore, the serological prevalence was 100% in the initial 30 samples from the herd. Based on the clinical symptoms and serological results, the Danish Veterinary and Food Administration (DVFA) on the 16 July decided to act in a precautious manner and kill the flock, which was carried out 19 July 2002. The virological examination became positive for APMV-1 with an ICPI of 1.71 on 2 August 2002.

This farm had reported no contacts to other farms, neither for the introduction nor the transmission of the infection. Strict bio-security measures were in place due to a previous infection with *Salmonella*. But a large colony of cormorants and herons existed in the vicinity of the farm and might have been a possible risk factor for passively transmission of ND virus through the ventilation system. One of the ventilators had been out of order and during repair it was put on suction instead of exhaustion. The poultry near this ventilator were the first ones to show clinical symptoms. The colonies of cormorants were tested for ND by sampling one cormorant, which was found dead, as well as manure collected below the nests, but no virus was isolated.

Table 3. Information on Newcastle Disease in commercial flocks

Outbreak, serial no.	Type of flock	Outbreak confirmed*	Diagnostic aspects	Source of infection
1	Pullets	26/07-02	Clinical: Ataxia, lameness, torticollis Serology 19/07-02	Primary introduction of unknown origin**
2	Layers	26/07-02	Clinical: Egg drop, lameness, torticollis Serology 20/07-02 Virology 26/07-02 ICPI = 1,75	Pullets from outbreak no. 1 15/06-02
3	Layers	26/07-02	Clinical: Ataxia, lameness, torticollis Serology 20/07-02	Pullets from outbreak no. 1 18/06-02
4	Layers	26/07-02	Clinical: Ataxia, torticollis Serology 20/07-02	Pullets from outbreak no. 1 15/06-02
6	Dealer flock, mixed poultry	26/07-02	Clinical: Lameness, Serology 20/07-02	Pullets from outbreak no. 1 28/05-02
7	Dealer flock, mixed poultry	26/07-02	No clinical symptoms Serology 20/07-02	Pullets from outbreak no. 1 13/06-02
28	Layer First suspicion of ND on 13/07-02	02/08-02	Clinical: Paralysis, egg drop Serology 15/07-02 Virology 02/08-02 ICPI= 1,71	Primary introduction of unknown origin**
29	Breeders	29/07-02	No clinical symptoms Serology 27 and 29/07-02 with a difference in titres	Primary introduction of unknown origin**
127	Layer	19/08-02	Clinical: Torticollis, egg drop Serology 20/08-02 Virology 04/09-02 ICPI= 1,75	Primary introduction of unknown origin**

* Outbreaks no.1, 2, 3, 4, 6, 7 and 28 were depopulated before the outbreaks were confirmed.
** Outbreaks no. 1, 28, 29 and 127 were all located less than 2 km from a coastline

The second suspicion (outbreak no. 1 situated nearby Hadsund in North-Jutland) had restrictions imposed on 18 July 2002.The suspicion was supported on 19 July 2002, as the flock showed the same serological pattern, as the first suspicion (100% prevalence in 60 samples and high titres up to 2048) in a batch of pullets introduced on 28 June 2002, and furthermore revealed torticollis as seen on the cover page photographs. The flock was killed on 27 July 2002.

Outbreak no. 1 had a key role in the epidemic because of its many secondary contacts, which made it important to investigate the epidemiological pattern concerning this herd. Lameness was observed as the first clinical symptom on 4 June 2002. The clinical symptoms were not related to any introduction of poultry, as the last previous introduction was in January 2002 of day-old chickens. According to Directive 90/539/EEC the certificates stated, that the flocks of origin were free from clinical symptoms and not vaccinated with a live ND vaccine less than 30 days before collection of the hatching eggs, ruling out infection from vaccination.

The first clinical symptoms were suspected to be due to Marek´s disease. During June 2002, material for virological and histological investigations was sent to DVI, but the investigations were inconclusive. On 7 August 2002 a RT-PCR test was found positive for Marek´s disease at DVI, from a sample of 10 pullets. On the owner's own initiative samples were also sent to a laboratory in Germany and were found positive for Marek´s disease on 22 July 2002. Furthermore, the German laboratory routinely tested the samples serologically for ND and found them seropositive on 18 July 2002. The owner reported this results to the RVC, who imposed restrictions on the herd and initiated the above-mentioned sampling of the flock.

APMV was never isolated from poultry from outbreak no. 1. Apparently the clinical symptoms of Marek´s disease in outbreak no. 1 shaded the clinical suspicion of ND and in combination with difficulties in isolation of the virus from the first suspicion (outbreak no. 28) these factors delayed the confirmation of the first suspicions of ND. The source of infection in outbreak no. 1 is still unknown.

Before restrictions were imposed on the farm, a regular sale of pullets from the previous batch had taken place from this farm to six contact flocks (outbreaks no. 2, 3, 4, 5, 6, 7), since 28 May 2002.

Poultry in outbreaks no. 2, 3, 4, 5 and 6 had shown varying degrees of clinical symptoms. Restrictions were imposed on all six contact flocks on 19 July 2002. Serological tests of these flocks revealed serologically positive reactions for ND on 20 July 2002 (titres up to 4096). Among these were the two poultry dealers (outbreak no. 6 and 7) who had resold pullets to a large number of back-yard flock keepers.

The dealer flock in outbreak no. 6 had resold all the purchased pullets, however turkeys on this farm were found to be seropositive for ND. The second dealer flock (outbreak no. 7) and his contact flocks, did not reveal any clinical symptoms, neither did the majority of contact flocks to the other dealers flock (outbreak no. 6).

From outbreak no. 2 it was reported, that pullets received on 15 June 2002 from outbreak no. 1 showed clinical symptoms at the receipt. APMV-1 with an ICPI of 1.75 was isolated from poultry in this outbreak on 26 July 2002. APMV was only isolated from poultry with origin in outbreak farm no. 2, and not from poultry with origin in outbreak farm no. 1. This indicates that outbreak no.1 had passed the acute phase of the infection in July, which can be the reason why the virus

could not be isolated from poultry in this outbreak. However, the infection was apparently transmitted with pullets from outbreak no. 1 to the other pullets already present on the farm in outbreak no. 2, as this flock developed clinical symptoms (lameness, egg drop) 3 weeks after introduction of the pullets from outbreak no. 1. As pullets from the original flock in outbreak no. 2 were in the acute phase of infection when samples were taken out, isolation of APMV was possible from these birds.

This was one of the few outbreaks where an active spread of the infection was observed. Both pullets originating from outbreak no. 1 and the original pullets in outbreak no. 2 were serologically positive, with titres ranging from 64 to 1024 and a prevalence of 100% in 60 samples.

On the farms in outbreaks no. 5 and 7, other flocks of poultry were kept in close contact with the pullets purchased from outbreak no. 1, but even though they had been in contact for more than one month, these other flocks were all serologically negative and did not show any clinical symptoms.

As demanded in the Danish regulations, the dealer in outbreak no. 7 had kept the compulsory records of all persons (17 back-yard flock keepers) to whom he had sold pullets originating from outbreak no. 1. Restrictions were imposed on these 17 contact flocks on 22 July 2002 and they were tested serologically on 24 and 25 July 2002. All pullets origination from this dealers flock were seropositive, whereas pullets of other origin in these contact flocks were all seronegative. These contact flocks were all killed and destroyed during the period 25-29 July 2002.

In outbreak no. 6 no pullets originating from outbreak no. 1 remained on the premises. The flock in this outbreak consisted of a mixture of different species of poultry. The main business on this farm was trade with different species of poultry taking place both on site, at weekly fairs and at markets. The dealer explained that the first clinical symptoms were observed in the pullets bought from outbreak no.1 on 28 May 2002. After antibiotic treatment the clinical symptoms disappeared and he re-sold all the pullets. Approximately six weeks later young turkeys showed clinical symptoms and increased mortality. On 20 July 2002 the results of the serological tests showed that only the turkeys were seropositive with titres 64-512, while all other species of poultry in this flock were seronegative.

This dealer had not kept the compulsory records on his sales, including names and addresses of his customers, but he told that he had sold pullets and other species of poultry in small batches to many different back-yard flock keepers. Via local radio and television, people in the southern Jutland were requested to contact the RVC, if they had purchased poultry from this dealer after 28 May 2002. Furthermore tracing of contacts to this outbreak was done based on telephone records from the dealer. Although tracing back of contacts to this outbreak was very laborious, it was done successfully as owners of back-yard poultry responded with great enthusiasm to the request to contact the RVC.

In the resulting more than 100 positive back-yard contact flocks, various serological patterns were seen. In some flocks only pullets from outbreak no. 1 were seropositive, whereas in other flocks also poultry of different origin were seropositive. Different species of poultry were involved in outbreak no.6, but the majority of positive poultry were pullets.

Outbreak no. 29 was on a farm in Sønderjylland County, in the Southeastern part of Jutland. The suspicion arose due to routine serological testing for ND of breeding flocks. The serological test

was carried out on 27 July 2002, and reaction was found to APMV-1 with low titres (16-128) in 52 out of 60 samples (23 out of 30 in house no. 1, 29 out of 30 from house no. 2). Repeated sampling was carried on 29 July 2002, where 300 blood samples were collected. From house no.1 serological reaction for APMV-1 was found in 119 out of the 150 samples (titres from 16 to 256) and from house no.2 serological reaction for APMV-1 was found in 78 out of 150 samples (titres from 16 to 64). Due to the difference in prevalence between the two houses and thus a possible transmission of the infection from house no. 1 to house no. 2 it was decided on the 29 July 2002 to declare the farm as an outbreak, because of a reasonable suspicion of subclinical infection of ND.

All poultry on the farm were killed on 30 July 2002. Sampling and testing by virus isolation was carried out, but no virus was isolated. The farm was situated only a few hundred meters from the coast and wild waterfowl was often seen around the farm. One of the feed silos on the farm was not covered and it is possible that feed from this silo may have been contaminated from wild birds and caused the infection of the poultry.

Outbreak no. 127 arose on a farm with layers, situated on an Island in the County of West Zealand. The suspicion arose due to clinical symptoms (egg drop, torticollis). Restrictions were imposed on the farm on 16 August 2002 where also sampling for virology and serology was carried out. The serological results revealed very high titres (range: 16-4096) indicating an active ND-infection. Furthermore, the serological prevalence was 100% in 61 samples. Based of the clinical symptoms and the serological results it was decided to kill the flock, which was carried out 21 August 2002. On 4 September 2002 the virological examination became positive for APMV-1 with an ICPI of 1.75. The source of infection is unknown, but might have been related to wild waterfowls, as there are numerous waterfowls on the island.

4.3. Investigations in the wild bird population around the primary outbreak farms

Despite intensive epidemiological investigations in the four primary outbreaks farms, no common source of infection could be identified (i.e. contaminated feed, contact with infected areas, e.g.). However, these four farms all have in common, that they are located less than 2 km from a coastline, with a potential risk of infection from wildlife waterfowl. With the objective to examine the possible presence of ND virus in the wild bird populations in costal areas in the vicinity of the infected farms, 1730 fresh faecal samples were collected for virologically examination during September and October 2002 (170 cormorants, 445 crows, 145 geese, 970 gulls). Two isolates of low-pathogenic APMV-1 was isolated from geese droppings. No ND viruses with similarities to viruses isolated from the infected farms were identified.

4.4. Serological methods and examinations

The Danish Veterinary Institute (DVI) is the National Reference Laboratory (NRL) for ND. The Department of Immunology and Biochemistry, Copenhagen, performs all serological analyses for ND in Denmark.

The serological method used is the Haemagglutination Inhibition assay (HI). The procedure applied at DVI is in accordance with Council Directive 92/66/EEC. Four HA units of virus (Strain: NDV Ulster 2C (15.02) 18.02.00) are used in the assay and accordingly titres above 8 are considered positive for antibodies APMV-1. The NRL, Department of Poultry, Fish and Fur Animals, Aarhus,

prepares all antigen and control sera used in the assay. HI analyses for antibodies against APMV-1 at DVI are accredited by Danish Accreditation (nr. 408) according to DS/EN/ISO/IEC 17025.

DVI has participated in the ring trials organised by the EU Commission's reference laboratory, with satisfactory results.

From 14 July to 20 December 2002, DVI has analysed a total of 37.794 blood samples from different species of poultry in relation to outbreaks, clinical suspect flocks, screening of contact and neighbouring flocks and other serological testing for ND. Serological reactions were found in 1683 of the samples with titres in the range: 16-4096. The samples originate from a total of 1.546 submissions to the laboratory. In 197 of the submissions, one or more positive reactions were found. Three submissions originated from flocks where low-pathogenic PMV-1 strains not related to the outbreak strain were isolated. The remaining 194 submissions with 1633 positive samples originated from 184 holdings. In 10 flocks, follow-up tests failed to identify ND virus and the flocks did not fit the outbreak criteria (non-outbreak flocks).

Figure 6 shows the distribution of the 1633 positive samples. The distribution has a mean of 389 and the following quartiles (Q): Q1=32, Q2=128 and Q3=512. In 135 flocks an outbreak was declared. During the post-outbreak screening programme, additional 39 flocks with sero-positive titres were identified (see below).

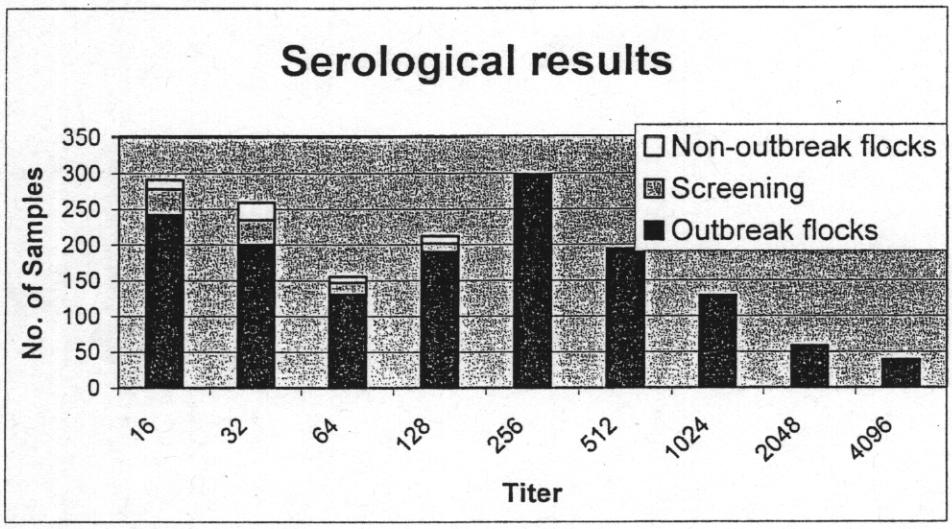

Figure 6. Distribution of positive samples detected during the ND outbreaks in Denmark in 2002

4.5. Virological methods and examinations

The Danish Veterinary Institute (DVI), as the National Reference Laboratory (NRL), performed the diagnostic work. The NRL, Department of Poultry, Fish and Fur Animals, Aarhus, performs all virological diagnostic procedures for ND in Denmark.

The diagnostic procedures for the diagnosis of ND were performed according to the methods laid down in Annex III of Council Directive 92/66/EEC and Commission Decision 95/117/EEC.

Samples from suspected flocks of poultry are inoculated into the allantoic cavity of 8-10 days-old specific pathogen free embryos of fowl. A total of 2 blind passages are made and the harvest from all eggs is tested for haemagglutinating activity. Haemagglutinating viruses are identified by haemagglutination inhibition (HI) test. Isolates of APMV-1 are further characterised by intracerebral pathogenicity index (ICPI) by inoculation in each of 10 SPF day-old chickens.

Although RT-PCR is not approved as a rapid diagnostic test it was applied as an additional test along with the approved procedures.

Until 6 November 2002 a total of 259 consignments related to the ND outbreaks or from poultry suspected of ND have been tested at DVI. Isolates of APMV-1 with an ICPI above 0.7 were found in three flocks.

Two isolates were forwarded to the EU Community Reference Laboratory at Weybridge, UK, for further typing and antigenic characterisation. Both isolates were identified as antigenic group C1 viruses, i.e. they belong to the same group as the virus strains, which caused ND in Denmark in 1995, 1996 and 1998, in UK and Ireland 1997, in Italy 2000 and which was also isolated in 2000, 2001 and 2002 from wild birds in Denmark.

Sequencing of the 2 isolates carried out at DVI, showed identical amino acid sequences at the cleavage site of the F protein and at a part of the F protein surrounding the cleavage site corresponding to 350 nucleotides of the viral genome. Thus, only one virus strain was involved in the present epidemic. Nevertheless, no evident epidemiological connection has been identified between the three flocks from which ND virus was isolated.

DVI experienced that the virus strain that occurred in 2002 was difficult to isolate as it grew very slowly at primary isolation and it may need blind passages before growing. Similar difficulties were not encountered with C1 group viruses isolated before 2002. Despite a considerable number of inoculations of various tissues from the three above-mentioned confirmed flocks as well as from suspected flocks, APMV-1 has so far only been isolated from brain tissue. Samples of tissues from diseased poultry in the last outbreak have been forwarded to the EU reference laboratory in Weybridge, who did not succeed in isolating virus from any of them. This confirms the difficulties by primary isolation of the virus strain.

5. Disease Control

5.1. Legislative matters

During the outbreaks a series of Orders were issued in order to regulate export and national transport of poultry, gatherings of birds, markets and exhibitions.

5.2. Crisis management structure

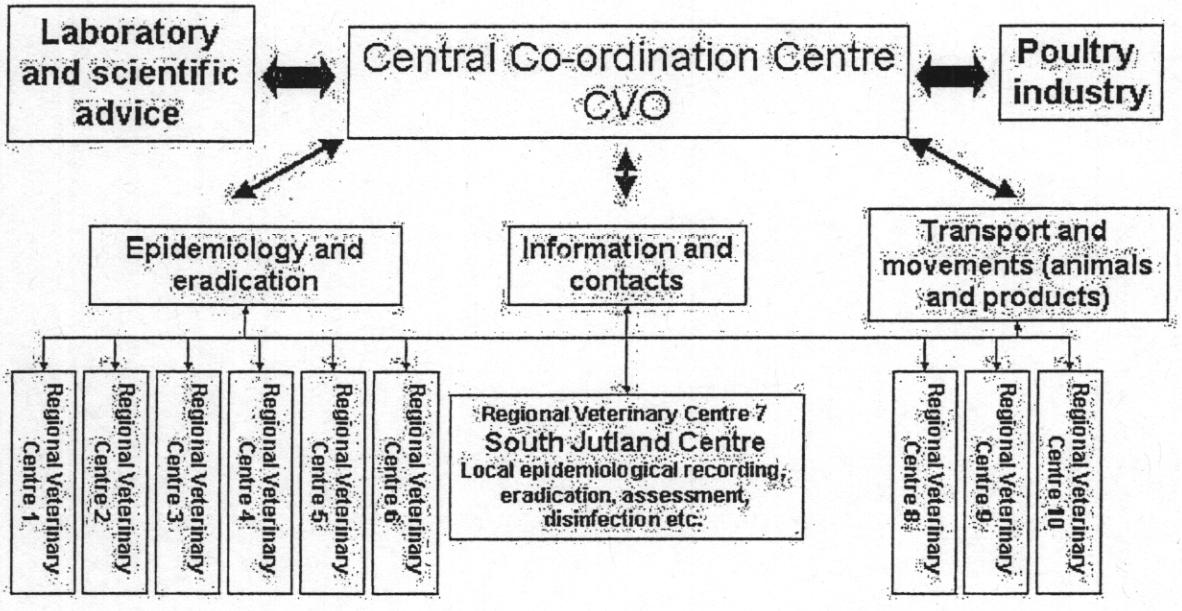

Figure 7. Crisis Management Structure applied during the ND outbreak

On 26 July 2002 when the first virological confirmation of ND was achieved, a Crisis Management Structure was established in order to optimise the coordination of the efforts to control the outbreak of ND. The Central Coordination Centre coordinated the activities in the three task force groups: Epidemiology and Eradication, Information and Contacts and Transport and Movements (Figure 7).

The Epidemiology and Eradication task force group coordinated the epidemiological investigations, the sampling of diagnostic material for testing, the eradication of infected poultry and the disinfection of infected holdings. The work was conducted in close corporation with the Local Crisis Centres, which were established at the RVC in the affected regions. Moreover, the Epidemiology and Eradication task force group was responsible for imposing surveillance and protection zones around each infected holding. A central GIS service was set up to provide maps and geographic information for the Local Crisis Centres to use in the eradication programme. Further, the location of ND surveillance- and protection zones were presented at a website. Due this service, the poultry industry and the DVFA personnel could locate any site in relation to current surveillance- and protection zones.

The Information and Contacts task force group continuously updated the European Commission on the outbreak and informed OIE on a weekly basis. A notification to the OIE took place as soon as the first official suspicion existed. Moreover, the Food and Agricultural Counsellors at Danish embassies were regularly updated on the ND situation with the objective to inform the Veterinary Administrations in a number of Third Countries. The task force group was also responsible for the administration of the Animal Disease Notification System (ADNS) and the update of the DVFA homepage in both Danish and English. This task force group was also responsible for giving information to the Ministry of Food Agriculture and Fisheries, the Danish Poultry Industry, other departments in the DVFA, the Veterinary practitioners and the media.

The Transport and Movement task force group was responsible for administrating the control of transport and movement, including export, of animals and products according to EU legislation and implementing Danish orders with ban on movement of poultry and products, to further reduce the risk of transmission of the infection. The task force coordinated the licensed transport and movement of animals and products within or between surveillance and protection zones in cooperation with the RVC's.

5.3. Measures in case of suspicion

According to Order No. 921 of 10 November 1994, the owner of poultry or other birds, suspected to be infected with ND virus, is obliged to call a veterinarian. The veterinarian is obliged to inform the RVC if ND infection is suspected and to take out samples for serological and virological examinations according to the instructions by the RVC. The RVC impose restrictions on the farm and on possible contact farms.

5.4. Measures in confirmed outbreaks

From the first positive serological test on 15 July 2002, due to precautionary reasons the DVFA based the establishment of protection and surveillance zones and the stamping out policy on:

1) Clinical symptoms and positive serology in commercial flocks or
2) Positive serology and contact (introduction of proven or suspected infected poultry) or
3) Repeated positive serology with a change in pattern of titres in paired flock samples.

Positive serology was used as an indicator of ND infection, as Denmark applies a non-vaccinating strategy for ND. The strategy with imposing restriction on the farm, establishing of zones and stamping out based on positive serology, either combined with clinical symptoms or contact to a confirmed outbreak, ensured the most rapid eradication of outbreaks and the immediate implementation of control measures. If stamping out had awaited confirmation by virus isolation these measures had been delayed and thereby the outbreaks could have become more widespread.

Thus, confirmation of the outbreaks in the 2002 ND outbreaks were based on:

1) Isolation and identification of virus as ND virus and determination of the pathogenicity of the virus using the ICPI test (outbreaks nos. 2, 28, and 127) or
2) Serial serological evidence (outbreak no. 29) or
3) Positive serology combined with either clinical signs or proven or suspected contact to other infected flocks or (all other outbreaks)

In confirmed outbreaks, measures according to Council Directive 92/66/EEC were imposed. All poultry on infected farms were killed and disposed by rendering or burying, followed by cleaning and disinfection of the farms.

5.5. Euthanisation

The euthanisation methods used varied according to flock size and the poultry species involved.

Exposure to carbon dioxide is listed in annex C of Council Directive 93/119/EEC as a possible killing method. This method was used to kill hens (domestic fowl of the species *Gallus gallus*) and turkeys. The animals were killed in a container, the size of which depended on the size of the flock. The carbon dioxide was supplied by a source of 100 % carbon dioxide, and the poultry were not introduced into the container until a sufficiently high concentration of carbon dioxide was reached. The speed with which the poultry were introduced into the container was adjusted to the supply of carbon dioxide. The poultry remain in the container until certain death.

Geese and ducks were killed by injection of a solution of pentobarbital sodium, as were small flocks of other poultry. This method was used for the killing of geese and ducks because of their reduced susceptibility to carbon dioxide. The pentobarbital sodium was administered as an intraperitoneal injection of a 30 % solution. The dose was 5 – 20 ml according to the size. After the injection the poultry were left without any disturbance until death had supervened.

5.6. Cleaning and disinfection

The requirements concerning cleaning and disinfection of infected holdings and lifting of protective measures applicable to the protection and surveillance zones, are provided by the provisions of the articles 9, 11 and Annex II of Council Directive 92/66/EEC.

As described below the key points as regard the cleaning and disinfection have been:

- the procedure for cleaning and disinfection as described in Annex II of Council Directive 92/66/EEC
- the cleaning and disinfection operations were carried out under official supervision
- the Regional Veterinary Officer carried out the approval of the preliminary and of the final cleaning and disinfection.

As soon as the killed poultry was transported to an approved rendering plant the initial cleaning and disinfection was initiated.

<u>The preliminary cleaning and disinfection in back-yard holdings</u>

All buildings and infected premises were disinfected with a 2% sodium hydroxide solution or Virkon®S. For disinfection of outdoor premises calcium hydroxide solutions were used. All disinfection solutions should stay on treated surfaces for at least 24 hours.

Manure and used bedding material was either burned or buried in the ground with a minimum of 30 cm depth.

Restrictions imposed on the holding remained in force.

The final cleaning and disinfection in back-yard holdings
After the preliminary cleaning and disinfection:

1. all visible dirt, manure etc. was removed,
2. all surfaces were treated with a degreasing agent,
3. all surfaces were washed with water and left to dry and
4. all surfaces were again disinfected with a suitable disinfection solution

Finally, points 2-4 were repeated.

The preliminary cleaning and disinfection in commercial holdings
All buildings and infected premises were disinfected with a 2% sodium hydroxide solution, Virkon®S or formaldehyde fumigation. For disinfection of outdoor premises calcium hydroxide solutions were used. All disinfection solutions stayed on treated surfaces for at least 24 hours. Restrictions imposed on the holding remained in force.

After the preliminary disinfection the following procedures were carried out:

1. Securing appropriate supply of equipment and water for cleaning purposes
2. Establishing a slurry container for contaminated cleaning water
3. Removal of manure to a covered stack
4. Dismantling of inventory and buildings
5. Burning of all materials that could be burned
6. Preliminary cleaning and disinfection
7. Washing of roofs
8. Final disinfection of outdoor premises
9. Final cleaning and disinfection of indoor premises
10. Disinfection/removal of slurry container
11. Approval of final cleaning and disinfection of indoor premises
12. Re-establishment of dismantled inventory and buildings

A risk assessment was carried out in order to decide when it was appropriate to assume that no virus could be spread from the infected premises.

The risk assessment included risk of transmission of ND virus by:

- Humans, as owners and cleaning and disinfection personnel, by securing appropriate cleaning and disinfection locks,
- Equipment and machines, by securing appropriate cleaning and disinfection procedures for material leaving the holding,
- Evaluating the preliminary cleaning and disinfection,
- Evaluating management of manure and wastewater.

5.7. Measures taken in protection and surveillance zones
Surveillance and protection zones were established according to Council Directive 92/66/EEC. During the entire period of the outbreaks, the epidemiological evidence from outbreak farms did not indicate any significant virus transmission from infected commercial or back yard flocks. The only transmission route documented for spread of infection between flocks during the outbreaks was trade with sub-clinically infected or sero-positive poultry, before the detection of the first outbreak.

As a general rule, it was therefore decided to establish minimum size surveillance zones (10 km), except in West Jutland and South Jutland, where large confluent zones were constructed (with success) to contain all subsequent secondary outbreaks. Protection zones were established without exceptions as minimum size (3 km) zones. The restrictions imposed in the zones and the lifting of the zones were in accordance with Council Directive 92/66/EEC.

5.8. Registration of poultry flocks

According to Danish legislation, commercial flock of poultry are registered in the 'Central Husbandry Register' database. Commercial flocks in protection- and surveillance zones were identified via the database.

Order No. 647 of 10 August 2002, issued due to the ND situation in Denmark, provided the legal power to require owners of hobby flocks to identify themselves, if they were located in risk areas.

The specific risk areas during the epidemic were: Nordjylland county south of Limfjord, the Counties of Viborg, Ringkøbing, Aarhus (5 named local communities), Vejle (4 named local communities), Ribe, Sønderjylland and the Sejerø island in West Zealand County.

Owners of hobby flocks had the possibility to register themselves via the DVFA homepage or by phone/mail to the RVC. From 9-13 August 2002, announcements about the registration requirements were made in regional and national television and radio broadcast stations. On 13 August 2002, announcements about the requirement to register hobby flocks were published in regional newspapers in the risk areas.

A total of 22,829 hobby flocks were registered on the basis of these efforts. Of these flocks 11.500 were situated within the surveillance zones and 3.433 in the protection zones.

5.9. Inspections and screening in protection zones

According to the Council Directive 92/66/EEC, a protection zone can be lifted when 21 days have elapsed since the preliminary cleaning and disinfection of the outbreak farm and an inspection and clinical investigation has been done in all poultry flocks in the zone.

This work was organised by the Local Crisis Centres, and took place during the end of August and the beginning of September. Commercial farms were inspected twice whereas backyard flocks commonly were inspected only once.

If the veterinary officer during the clinical examination of flocks within the protection zones detected clinical symptoms which could be indicative of ND among the poultry, restrictions were immediately imposed on the flock and the flock was serologically tested for ND.

In addition to the clinical examination, all poultry flocks situated within a 500 m zone from any outbreak were subjected to a serological ND test.

If the serological result was positive for ND, further samples were taken for virological examination and the flock was culled as a precautionary measure.

Figure 8 shows the strategy developed for inspection and diagnostic testing in protection zones.

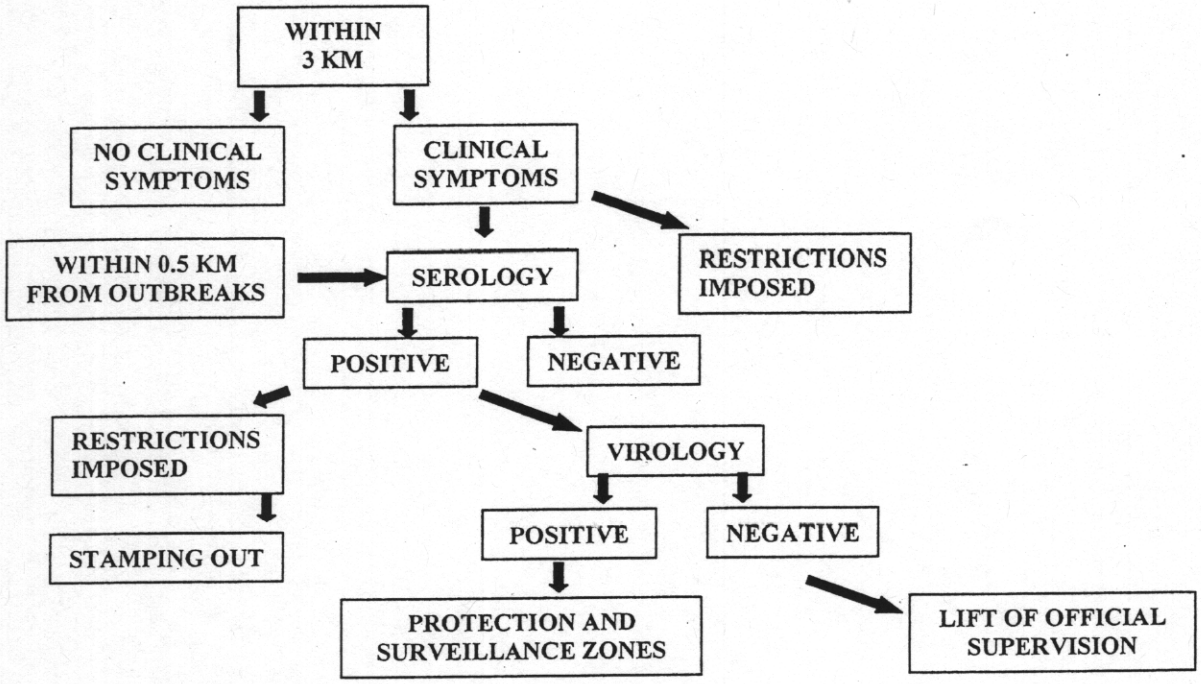

Figure 8. Strategy applied to inspection and screening in protection zones

5.10. Screening results

Table 5 shows the results of the serological screenings carried out in August –September. A total of 604 flocks were tested serologically. Of those tested, 39 contained one or more positive samples and were subjected to virological investigations. In four flocks, unspecific clinical symptoms of disease were the reason for sampling. Virological follow-up tests in all 39 flocks were negative.

Table 5. Clinical and serological screening of flocks in protection zones

County	Back-yard flocks	Protection zones	Flocks visited	Flocks tested	Positive flocks	Samples tested from positive flocks	Pos. samples
W. Zealand	164	1	22	6	0	-	-
S. Jutland	5215	100	2435	515	37	341	96
Ribe	3159	6	160	27	1	19	7
Vejle	1404	1	19	0	-	-	-
Ringkøbing	4086	20	619	50	1	12	2
Aarhus	1255	1	65	4	0	-	-
Viborg	4410	2	46	1	0	-	-
N. Jutland	2637	4	67	1	0	-	-
TOTAL	22330	135	3433	604	39	372	105

The distribution of the 105 positive serological titres from the screening appears from Figure 9. This can be seen to differ considerably from the distribution of titres found in earlier phases of the epidemic (see Figure 6).

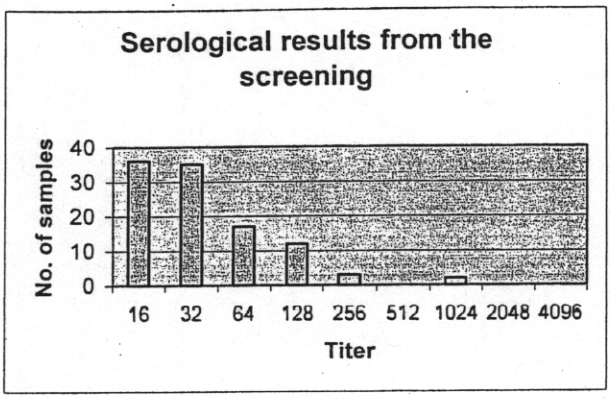

Figure 9. The distribution of the 105 positive serological titres from 39 flocks found sero-positive in the screening round in August-September 2002.

5.11. Regionalisation measures

Following the initial total export bans established by the Danish authorities for live poultry and hatching eggs, a series of six subsequent regionalisations were introduced. These decisions were directed to - and accepted by - the European Commission and the EU member states in order to gradually re-open the market for trade of live poultry and hatching eggs from Denmark.

Table 6 and Figure 9 describe the successive regionalisations, which were terminated with the lifting of all remaining restrictions as of 1 January 2003.

Table 6. Regionalisations for trade of live poultry and hatching eggs from Denmark within the EU.

Map	Date	Items	Description of open regions
Figure 9 - 1	19. Aug 2002	Poultry for slaughter and hatching eggs	Zealand, Funen and surrounding islands
Figure 9 – 2	29. Aug 2002	+ poultry for live use	+ North Jutland
Figure 9 – 3	12. Sep 2002	Same items	+ Central Jutland
Figure 9 – 4	2. Oct 2002	Same items	+ North-central Jutland except remaining surveillance zones
Figure 9 – 5	13. Oct 2002	Same items	+ South Jutland except remaining surveillance zones
Figure 9 – 6	2. Nov 2002	Same items	All of Denmark except two remaining surveillance zones
	1. Jan 2003	Same items	All remaining restrictions lifted

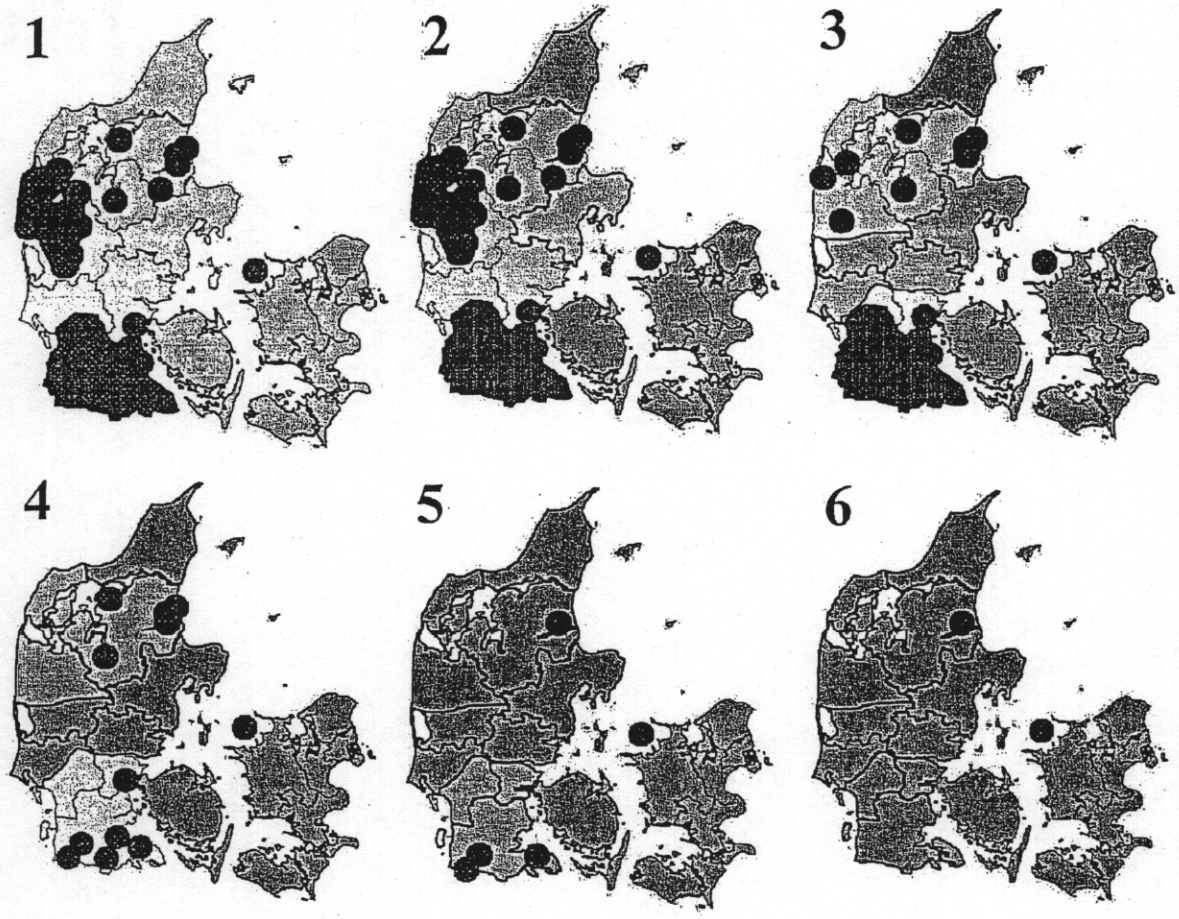

Figure 9. The series of six successive regionalisations. Sand-coloured areas indicate restricted regions. Blue-green areas show the location of existing surveillance zones, in which restrictions apply by default. The mint green areas show the new open regions. The grass green areas show the existing open regions. See Table 6 for further explanation.

5.12. Further information on ND surveillance during 2003

During 2003 a total of 15 cases of suspicion for ND were raised, which all turned out to be negative for ND.

Practicing veterinarians or the RVC raised 8 suspicions due to clinical symptoms, and 7 suspicions were raised by the NRL due to laboratory results, findings at the autopsy or the history of disease. The suspected cases involved 10 hobby flocks, 4 commercial flocks and 1 quarantine station for imported birds. Restrictions were imposed on all flocks when suspicions were raised and lifted when they turned out to be negative for ND.

All serological tests performed in 2003 due to the ongoing serological ND surveillance of the breeding flocks and the compulsory testing of pullets, the latter were imposed as a consequence of the 2002 ND outbreak, were negative.

6. Conclusions

The outbreaks of ND in 2002 in Denmark were atypical in the sense, that there were very limited clinical symptoms, there were only a few incidences of documented transmission of the virus within and between flocks, the virus was very slow and difficult to grow despite a high ICPI. It was only possible to isolate the virus from brain tissue, but not from the intestine or from the respiratory system. The latter might explain why transmission of virus was found infrequently, as the virus normally multiplicates in the intestinal and respiratory systems and is transmitted by faeces and respiratory secretions.

Only two primary outbreaks had a classical diagnostic course with suspicion due to clinical symptoms, seropositive poultry and isolation of virus. Due to lack of clinical symptoms particularly in back yard flocks, most diagnoses have been confirmed according to the criteria: positive serology and proven or suspected contact to a confirmed outbreak. As the serologically positive flocks revealed rather high serological titres, it would be rather unlikely, that the observed pattern should be due to illegal vaccination or exposure to low-pathogenic APMV.

Virological confirmation of the first suspicion was delayed due to problems with isolation of the virus. However, in spite of the absence of a virological diagnosis, Denmark applied the precautionary principle and acted at an early state as if ND had already been confirmed virologically. Restrictions were immediately imposed on the suspected flock and its contact flocks, and protection- and surveillance zones were established. Once the first outbreak was virologically confirmed on 26 July 2002 there was no delay in establishing the secondary outbreaks in contact flocks.

The epidemiological investigations in commercial flocks indicates that outbreak no.1 had passed the acute phase of the infection when it was recognised in July, which might be the reason why the virus was not isolated from poultry from this outbreak. However, the infection was apparently transmitted to poultry of other origin in outbreak no. 2, and as these pullets were in the acute phase of infection it was possible to isolate the virus from this outbreak. Apparently the clinical symptoms of Marek´s disease in outbreak no. 1 shaded the clinical suspicion of ND and in combination with difficulties in isolation of the virus from the first suspicion (outbreak no. 28) these factors delayed the first confirmation of an outbreak The massive spread of infected birds by the two poultry dealers (outbreaks nos. 6 and 7) to a large number of back-yard flocks caused the extent of the ND outbreaks in Denmark in 2002.

The high level of awareness of poultry keepers, resulting in the rapid registration of almost 23.000 back-yard flocks, was a very positive experience and contributed to an effective eradication of the disease.

Effective tracing, testing, culling, cleaning and disinfection measures were instigated by the authorities, so that in spite of the relative large epidemic, the national eradication was more or less completed within a 6 week-period. The successful eradication was further documented by an extensive screening program within the protection zones, which was completed in mid-September. Furthermore, the resulting series of 6 successive regionalisations were readily accepted by the European Commission and by the other EU member states.

As a consequence of the ND outbreaks Denmark has imposed compulsory serological testing of pullets for ND. Movement of pullets from breeding farms to laying farms is now allowed only if serological tests for ND without any reactions in the flock have been carried out. Additional changes to contingency plans have also been decided, so that valuable experiences from the ND epidemic will be of benefit to any future eradication and control situation, which may occur. Furthermore, it has been decided to carry out a campaign among poultry dealers in 2004, to ensure that they record and keep the compulsory information about the purchasers of all traded poultry. Finally, the ND-non-vaccination policy is currently under discussion.