Acta vet. scand. 1985, *26*, 521—532.

From the National Veterinary Institute, Uppsala, Sweden.

# PATHOGENICITY FOR CHICKENS OF A PARAMYXOVIRUS TYPE 1, ISOLATED FROM RACING PIGEONS IN SWEDEN

By

*Björn Engström, Oddvar Fossum* and *Martin Wierup*

ENGSTRÖM, BJÖRN, ODDVAR FOSSUM and MARTIN WIERUP: *Pathogenicity for chickens of a paramyxovirus type 1, isolated from racing pigeons in Sweden.* Acta vet. scand. 1985, *26*, 521—532. — Strains of paramyxovirus type 1 (PMV-1) have been isolated from diseased racing pigeons in Sweden. One of these isolates was selected for studies of the pathogenicity and contagiousness in chickens.

The same isolate was previously found to have a high intravenous pathogenicity index (IVPI) in 6 weeks old chickens.

In three experiments it was found that the PMV-1 isolate was very pathogenic for 1 week old chickens but not pathogenic for 120 day old pullets inoculated intranasally and ocularly.

Symptoms in the young chickens were similar to those seen in the neurotropic form of Newcastle disease. The mortality was high and the incubation period 5—11 days.

The disease easily spread to young chickens kept in contact with diseased birds.

The microscopic examination revealed an interstitial nonpurulent pneumonia and a nonpurulent encephalitis in the young chickens. In the pullets the only finding was a mild encephalitis.

PMV-1 was recovered from all young chickens but not from the pullets.

Both the chickens and the inoculated pullets developed antibodies to PMV-1.

intranosal-ocular inoculation; PMV-1; high mortality; horisontal transmission; immune response; haemagglutination inhibition test; encephalitis; pneumonia.

During the autumn of 1983 outbreaks of an acute disease appeared in flocks of racing pigeons in the south of Sweden.

The symptoms were diarrhoea in the acute stage and torticollis and paralysis in the birds surviving the acute stage.

A disease in racing pigeons similar to this was reported during 1983—84 from many European countries (*Alexander et al.*

1984 a). Viruses isolated from diseased racing pigeons were identified as paramyxovirus type 1 (PMV-1) (*Biancifiori & Fiorni* 1983, *Viaene et al.* 1983, *Richter & Kösters* 1983, *Alexander et al.* 1984). Many PMV-1 isolates from racing pigeons in different parts of Europe were shown to be closely interrelated and could be distinguished from paramyxovirus type 1 strains previously isolated from outbreaks of Newcastle disease in poultry (*Alexander et al.* 1984 a).

Experimental infections with several isolates have been performed in both pigeons and chickens.

*Alexander & Parsons* (1984) found that pigeons infected by the intranasal route did not show any clinical signs of disease, but they excreted virus in faeces and infected pigeons by contact. Chickens, 3 weeks of age, in contact with infected pigeons showed an immune response and excreted virus in faeces, but did not show any clinical signs of disease. When the same isolate 561/83 was inoculated intravenously in 6 week old chickens 3 out of 10 birds died within 10 days.

*Viaene et al.* (1983) inoculated pigeons intranasally and found that the pigeons first got diarrhoea and later nervous symptoms. Chickens in contact with these pigeons did, however, not show any sign of disease. In another experiment by *Viaene et al.* (1983) 10 one day old chickens were infected intranasally and ocularly. Three of these birds became paretic.

*Biancifiori & Fioroni* (1983) infected pigeons intranasally and ocularly. All pigeons showed nervous and respiratory signs of disease and the mortality was high. In several experiments chickens of different age, were inoculated by several routes. None of the chickens showed any signs of disease in these experiments.

The virulence and transmissibility in chickens and pullets of a Swedish paramyxovirus type 1 isolate are studied in this work.

## MATERIALS AND METHODS

### *Virus*

Viruses were isolated by Dr G. Rockborn, National Veterinary Institute, Uppsala from 4 outbreaks of disease with torticollis and paralysis in racing pigeons. All 4 isolates were classified by Dr. Alexander, Central Veterinary Laboratory, Weybridge, Great Britain and were found closely related to paramyxovirus type 1 (PMV-1) isolated from racing pigeons throughout Europe. In

connection with the identification Dr. Alexander performed pathogenicity tests (*Alexander et al.* 1985) with two of the Swedish isolates.

Isolate Vi 2602/83, which was selected for this study, had an intracerebral pathogenicity index (ICPI) of 1.58 and an intravenous pathogenicity index (IVPI) of 1.81. This isolate was from the second passage through embryonated fowls' eggs. The same passage was used in the experiments described in this paper.

*Serological tests*

Haemagglutination inhibition tests were done in V-bottomed plastic microtitre plates using doubling dilutions, 1 % chicken red blood cells, 4 haemagglutinating units of virus and 0.05 ml volumes. Sera were heated at 56°C for 30 min before use. Titres were expressed as the reciprocal of the highest dilution of serum causing inhibition of 4 haemagglutinating units of virus. Isolate Vi 2062/83 was used as test virus.

*Virus isolation*

Specimens for virus isolations were collected from the chickens in the 3 experiments. Samples of intestine and pooled trachea and lung were made into a 10 % suspension in phosphate buffered saline (PBS) pH 7.2. Of these suspensions 0.2 ml were inoculated into the allantoic cavity of 10-day-old embryonated fowls' egg. Presence of paramyxovirus was indicated by haemagglutination test.

*Histopathology*

Specimens were taken from brain, lung, trachea, spleen, bursa of Fabricius, intestine and pancreas and fixed in 10 % formalin, processed by standard paraffin tehcniques and sections stained with haematoxylin and eosin (HE).

*Experimental design*

In order to study the pathogenicity of the PMV-1 isolate Vi 2602/83 for chickens of different ages, 3 experiments were set up. In all experiments the birds were kept in isolators with wire-netting floors and the air was filtered through HEPA-filters. Birds were inoculated with $10^8$ 50 % egg infectious doses of virus Vi 2602/83 by both the intranasal and ocular route. Some birds

were also placed in contact with the infected ones to study the transmissibility of the disease.

The design of the experiments is shown in Table 1.

Table 1. Design of 3 experimental infection trials with paramyxovirus type 1 isolate Vi 2602/83.

| Experiment Number | Age at infection (days) | Number of birds | | Age at contact (days) | Origin of birds |
|---|---|---|---|---|---|
| | | Infected | Controls | | |
| 1 | 9 | 50 | 25 | 9 | Commercial |
| 2 | 7 | 18 | 6 | 8 | Specific pathogen free |
| 3 | 120 | 6 | 4 | 123 | Commercial |

In Experiment 1 the contact birds were present in the isolator when the other birds were inoculated and some of them might then have been infected by aerosol. In order to avoid infection of the contact birds during the inoculation procedure in Experiments 2 and 3 the contact birds were introduced 1 and 3 days respectively after the inoculation. Commercial chickens in Sweden are free from Newcastle disease (ND) and vaccination is not allowed.

Blood samples for serological examination were collected during the experiment at different intervals. Diseased birds were killed when they became moribund.

Samples for virus isolation were taken from all dead and killed birds.

## RESULTS

### *Clinical observations*

Experiment 1. All infected and most of the contact birds showed signs of disease 1—2 weeks after inoculation. The symptoms were general depression and in the severe cases paralysis, tremor, opistotonus and torticollis.

The incubation period was 5—11 days for the inoculated birds. The contact birds were less severely affected, but fell ill at the same time as the inoculated birds. Some of the birds were only depressed and were killed at 37 days of age, when the experiment finishtd. At that time these birds looked like so called "helicopter chickens" with broken feathers sticking out from

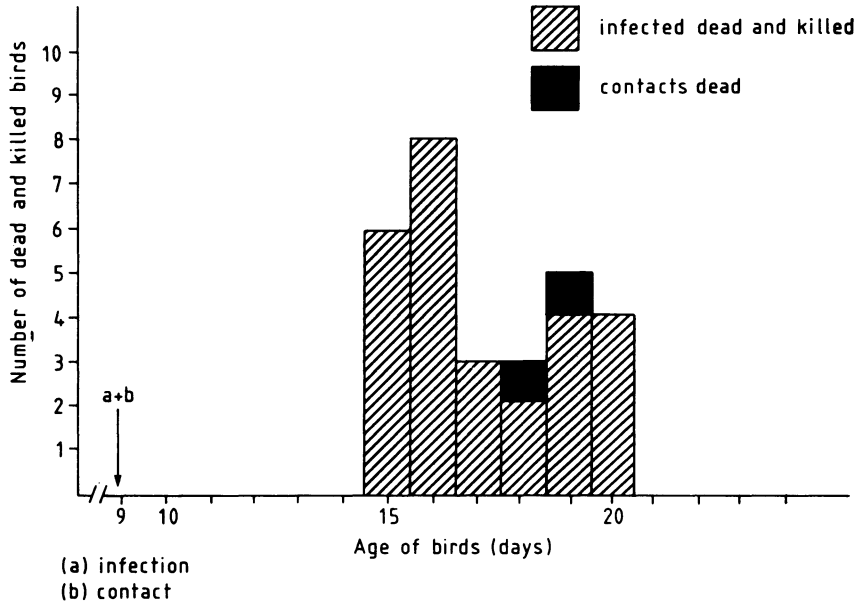


Figure 1. Experiment 1: Paramyxovirus type-1 isolate Vi 2602/83 inoculated intranasally and ocularly in 50 nine-days-old chickens with 25 chickens present as contact birds. Moribund birds were killed. Seventeen birds were killed during the experiment for virus isolation and 27 were killed at the end of the experiment.

the body. Twentyseven of the 50 infected birds and 2 of the contact birds died or were killed when they became moribund (Fig. 1). Seventeen inoculated birds without severe clinical symptoms were killed during the experiment for virus isolation and blood sampling.

Experiment 2. In this experiment all inoculated birds and all except one of the contact birds died or had to be killed (Fig. 2).

The symptoms were the same as in Experiment 1. The incubation period was 5—7 days for the inoculated birds and 6—9 days after the onset of disease in these birds the contact birds fell ill.

Experiment 3. This experiment was an attempt to reveal the susceptibility of older chickens to PMV-1.

In this experiment none of the pullets showed any signs of disease. The inoculated birds were killed 13 and 22 days after infection and the contact birds 19 and 24 days after contact.

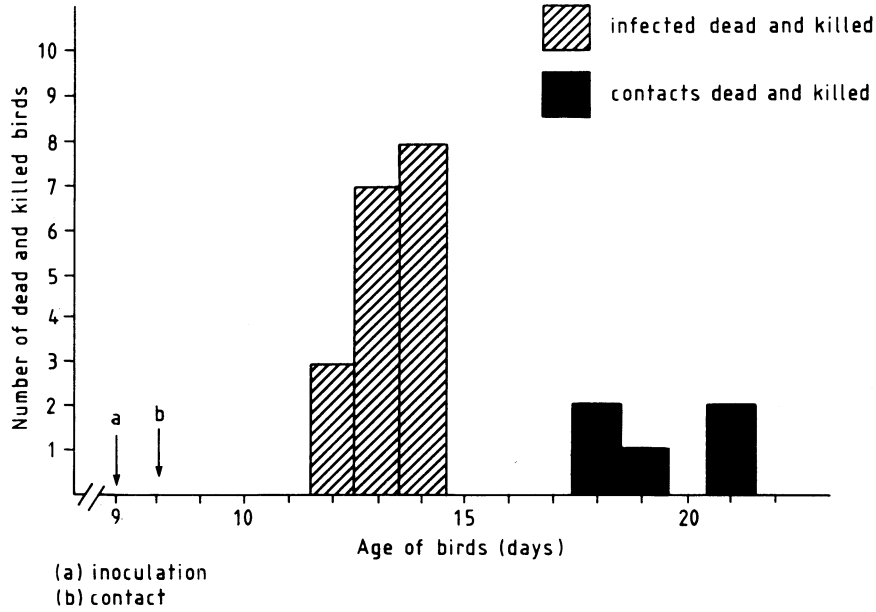


Figure 2. Experiment 2:Paramyxovirus type-1 isolate iV 2602/83 inoculated intranasally and ocularly in 8 seven-days-old SPF chickens. Six SPF chickens were moved to the isolator the next day and served as contact birds. All the infected and contact birds died or had to be killed and only 1 of the contact birds survived without severe symptoms of disease.

*Pathology*

Gross lesions. In Experiments 1 and 2 a small properion of the birds had ecchymotic haemorrhages in the gizzard underneath the keratinoid layer and petechial haemorrhages in the mucosa of the proventriculus.

In Experiment 3, five out of 6 inoculated pullets and 2 out of 4 contact birds had extensive haemorrhages in the cecal tonsils and in the intestinal mucosa next to the tonsils. Petechial haemorrhages in the proventiculus were seen in 1 of the inoculated birds.

Microscopic lesions—Experiments 1 and 2.

Brain: In all the chickens (both inoculated and contact birds) perivascular and even diffuse infiltrations of mononuclear cells were demonstrated as well as gliosis and neuronal degeneration (Fig. 3).

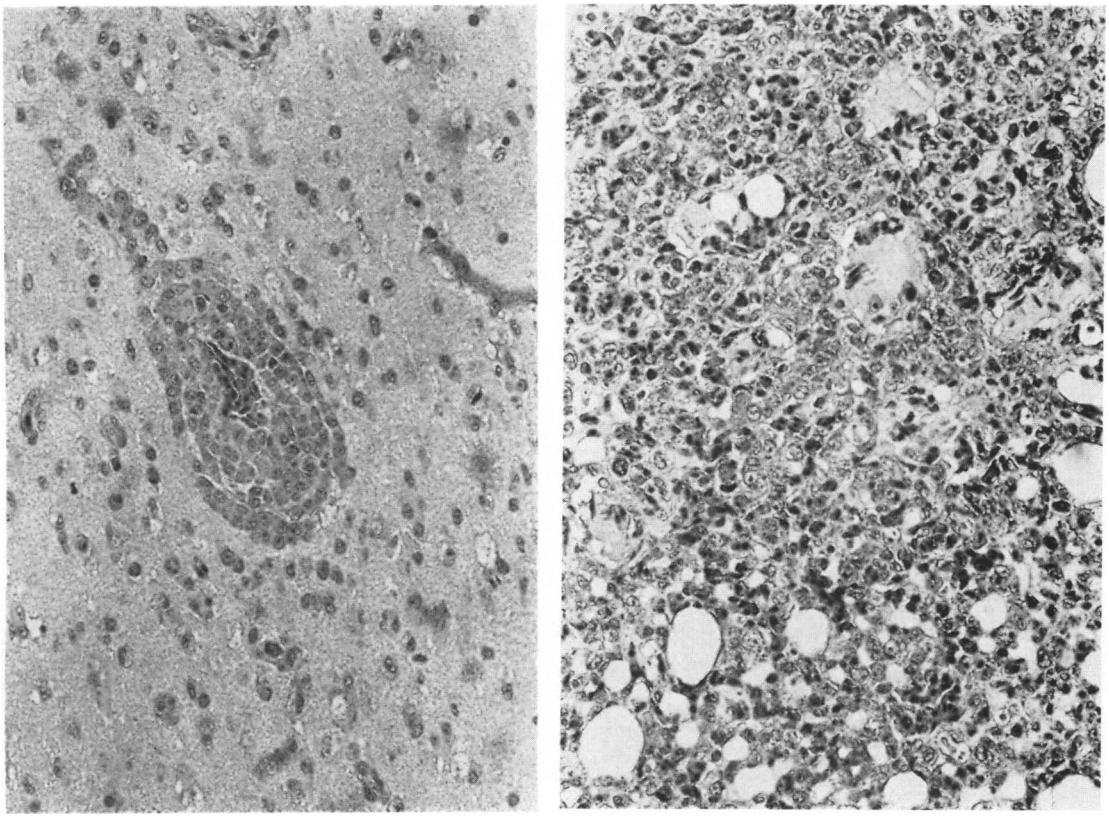

Figure 3. Brain from inoculated chicken 7 days after inoculation showing perivascular infiltration of monocuclear cells. H & E, x 175.

Figure 4. Lung from chicken 6 days after inoculation showing thickening of the interatrial and interparabronchial walls. Furthermore, oedema, hyperemia and invading mononuclear cells can be seen. H & E, x 175.

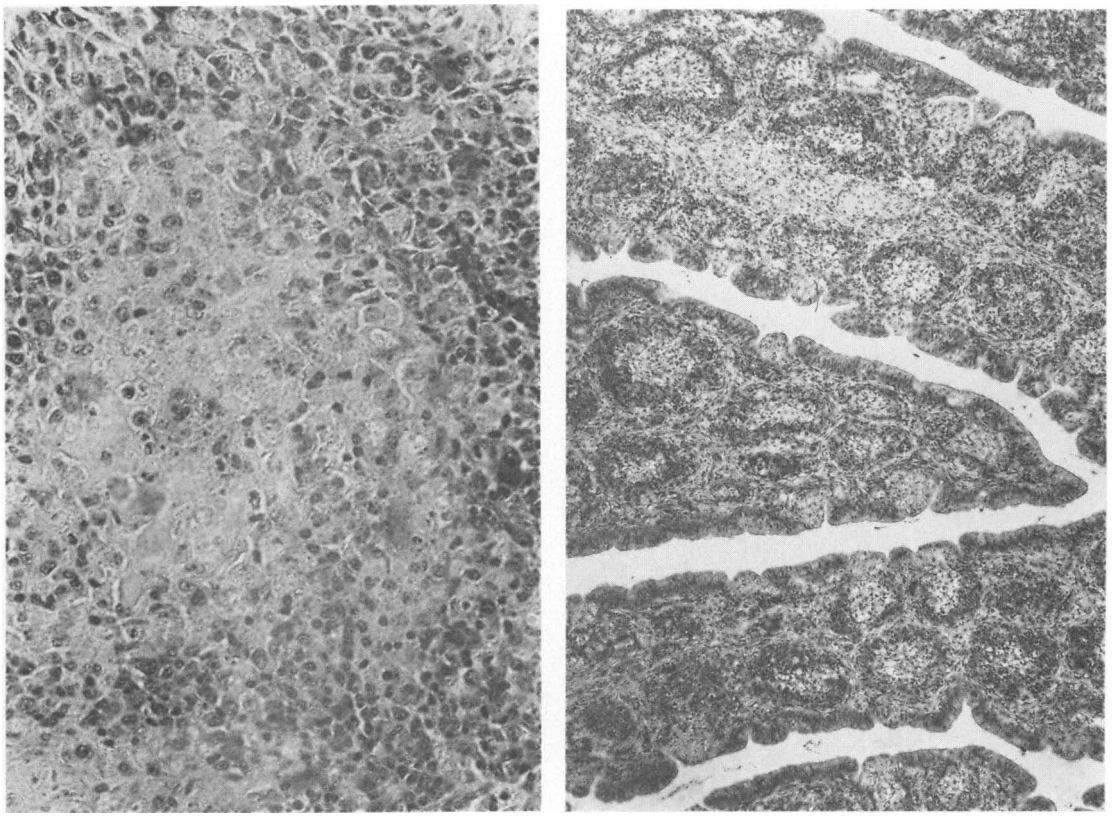

Figure 5. Focal necrosis in spleen from inoculated chicken 5 days after inoculation. H & E, x 175.

Figure 6. Bursa of Fabricius from inoculated chicken 6 days after inoculation. Extensive hyperplasia of interfollicular connective tissue. The follicles are small and medulla is almost completely depleted of lymphocytes. H & E, x 45.

Table 2. Immune response in birds infected experimentally with paramyxovirus type 1 isolate Vi 2602/83.

| Experiment Number | | Days after infection or contact | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 8 | 9 | 11 | 12 | 13 | 15 | 17 | 18 | 19 | 20 | 22 | 28 | |
| 1 | Infected | 10* 1** | | | | | | | 10 5.9 | | | | 8 6.4 | | 9 6.6 | |
| | Contact | 8 1 | | | | | | | 8 5.8 | | | | 8 6.0 | | 8 6.1 | |
| 2 | Infected+ | 10 1 | | | | | | — | | — | — | | | | | |
| | Contact | 6 1 | 6 1 | | 4 2.5 | | 2 4.5 | | | 1 6.0 | | | | | | |
| 3 | Infected | 6 1 | | 6 4.2 | | | | 6 4.7 | | | 4 6.0 | | | 4 6.8 | | |
| | Contact++ | 4 1 | 4 1 | | | 4 2.0 | | | 4 3.0 | | | 4 3.0 | | | | |

*) Number of birds tested
**) Mean $\log_2$ haemagglutination inhibition titre of group
+) All birds died early after inoculation
++) Only one bird showing immune response

Lung: In almost all the chickens thickening of the interatrial and interparabronchial septa was seen. There was a hypertrophy and hyperplasia of the cells of the alveolar walls and a diffuse invasion by mononuclear cells. Congestion and lung oedema were also common findings (Fig. 4).

Trachea: One case of catarrhal tracheitis was seen.

Spleen: In most of the chickens multiple, small, hyaline necroses were demonstrated (Fig. 5).

Bursa of Fabricius: Extensive degeneration of lymphocytes, lymphocyte depletion and thickening of the connective tissue strands were observed in all the chickens (Fig. 6).

Pancreas: Massive infiltrations of mononuclear cells were common findings.

Experiment 3.

*Brain:* In both inoculated and contact pullets there were perivascular infiltrations of mononuclear cells and gliosis. No obvious signs of neuronal degeneration were observed. No significant histophatological lesions were recorded in other organs.

*Serology*

The results of the serological examination are shown in Table 2. All tested birds in Experiments 1 and 2 showed an immune response. In Experiment 2 all the inoculated birds died or had to be killed after 1 week and only the contact birds could be tested serologically. In Experiment 3 all the inoculated birds, but only 2 of the contact birds showed an immune response. The immune response was similar in all experiments. The first HI-antibodies were detected 1 week after infection and the titres were rising until the end of the experiments.

*Virus isolation*

In Experiments 1 and 2 PMV-1 was isolated in all birds from both intestine and from pooled lung and trachea samples. In both Experiments 1 and 2 the last birds were killed 28 days after infection and they still carried virus.

In Experiment 3, PMV-1 was not recovered from any bird. The first birds were killed 13 days after infection and the last one 9 days later.

## DISCUSSION

Paramyxovirus type 1 serogroup of avian paramyxoviruses (PMV-1) have been isolated from racing pigeons in Sweden and many other European countries with a disease resembling the neurotropic form of Newcastle disease (ND) in chickens.

Many of these isolates have been shown to be closely interrelated by serological tests using monoclonal antibodies. They could also be distinguished from paramyxovirus type 1 isolated from outbreaks of ND (*Alexander et al.* 1974 a).

Several experiments have revealed that it is possible to transmit the disease to pigeons with pigeon PMV-1 isolates by intranasal and ocular inoculation (*Viaene et al.* 1983, *Biancifiori & Fioroni* 1983).

As the pigeon PMV-1 isolates belong to the same serotype of PMV as Newcastle disease virus (NDV), it is of great importance to study the pathogenicity of these isolates for chickens. In previous experiments with pigeon PMV-1 isolates in chickens, most chickens did not show any signs of disease when infected either by pigeons shedding the virus or when inoculated intranasally and ocularly (*Alexander et al.* 1984 a, *Viaene et al.* 1983, *Biancifiori & Fioroni* 1983). Intravenous inoculation of 6 weeks old chickens (IVPI-test) revealed a very varying pathogenicity between different isolates (*Alexander & Parsons* 1984). The conclusion of these experiments was that pigeon PMV-1 was not very pathogenic to chickens. In the beginning of 1984, however, a closely interrelated PMV-1 strain caused several outbreaks in poultry farms in England (*Anon.* 1984). *Alexander et al.* (1984b) later found that a fowl isolate from the first of these outbreaks killed 2 out of twelve 2 year-old chickens after ingestion. This isolate was already well adapted to chickens.

In the present experiments with isolate Vi 2602/83 chickens of different ages were inoculated with an equal dose and the same route of infection as in some of the experiments quoted above. Allantoic fluid from the second passage through embryonated fowls' eggs were used. The PMV-1 isolate appeared to be very virulent in young chickens and was easily transmitted to non infected chickens kept in contact with the infected ones. The symptoms were as seen in the neurotropic form of ND. The incubation period was only 5—11 days for the experimentally infected birds and the control birds fell ill after about the same

period of time after the first sign of disease in the inoculated ones in Experiment 2. In Experiment 1 there were no separate outbreak in the contact birds. This can be explained by the fact that these birds were present during the inoculation procedure and then become infected with a low dose of virus.

The mortality was very high in the young chickens. The pullets were, however, more resistant and did not show any signs of disease during the whole experiment. Shedding of the virus was apparently limited in the pullets as only 1 of the contact birds seroconverted.

The neurotropic nature of the isolate was verified by the histopathological examination. All birds in these experiments had a nonpurulent encephalitis. Most of the young chickens also had an interstitial pneumonia even though respiratory signs were not seen as the nervous symptoms dominated completely. The lesions in spleen and bursa of Fabricius reflect a destruction of lymphoid cells also typical for ND. Isolate Vi 2602/83 was consequently as neurotropic in chickens as in the spontaneous cases in pigeons. Virus was regularly isolated from lung, trachea and intestine in the experiment with young chickens until the end of the experiment. In the older birds, however, it was not possible to recover virus 13 days post infection from these tissues. The replication of virus is probably more limited in older birds.

All surviving chickens and all infected pullets showed an immune response with maximum haemagglutination inhibition titers of 1:64—1:128 against Vi 2602/83 three weeks after infection. This corresponds very well with previous reports.

The isolate Vi 2602/83 was obviously more pathogenic for chickens infected by natural routes than other previously described pigeon PMV-1 isolate. The IVPI of this isolate was also one of the highest in a comparative study performed by *Alexander et al.* (1985). It would therefore have been interesting to study the pathogenicity of other European isolates with different IVPI values in experiments with chickens inoculated intraocularly and intranasally at different ages as in this study.

The difference in pathogenicity is difficult to explain. The pigeon PMV-1 is, however, likely to be a variant strain of NDV with a varying degree of adaptation to pigeons. *Alexander et al.* (1985) discuss how passage through chickens or chick embryos may result in virus more virulent for chickens. This can explain why our 120 days old chickens did not fall ill while 2-year-old

chickens infected with a fowl isolate (*Alexander et al.* 1984 b) were more susceptible.

The risk for transmission of the virus to poultry is obvious. The result of our experiments and the experience of clinical outbreaks in England (*Anon.* 1984) show that the pigeon PMV-1 is a potential pathogen for poultry.

Sweden is so far free from ND in poultry. There has not been any signs of infection with pigeon PMV-1 in the poultry production and a recent serological survey revealed no subclinical cases either. Vaccination against ND is not allowed in poultry. However, all racing pigeons in Sweden are now vaccinated with a killed PMV-1 vaccine in order to limit further spread of the infection.

## ACKNOWLEDGEMENTS

The authors wish to thank Mrs. I. Lif and Mrs. E. Forsgren-Mörth for excellent technical assistance and Mrs. M. Olde for typing the manuscript.

This research was supported by a grant from the Swedish National Board of Agriculture.

## REFERENCES

*Alexander, D. J., P. H. Russell & M. S. Collins:* Paramyxovirus type 1 infection of racing pigeons: 1. Characterisation of isolated viruses. Vet. Rec. 1984 a, *114*, 444—446.

*Alexander, D. J., G. Parsons & R. Marshall:* Infection of fowls with Newcastle disease virus by food contaminated with pigeons faeces. Vet. Rec. 1984 b, *115*, 601—602.

*Alexander, D. J. & G. Parsons:* Avian paramyxovirus type 1 infections of racing pigeons: 2. Pathogenicity experiments in pigeons and chickens. Vet. Rec. 1984, *114*, 466—469.

*Alexander, D. J., P. H. Russell, G. Parsons, E. M. E. Abu Elzein, A. A. Ballouh, K. Cernik, B. Engström, M. Fevereiro, H. J. A. Fleury, M. Guittet, E. F. Kaleta, U. Kihm, J. Kosters, B. Lomniczi, J. Meister, G. Meulemans, K. Nerome, M. Petek, S. Pokomunski, B. Polten, M. Prip, R. Richter, E. Saghy, Y. Samberg, L. Spanoghe & B.Tumova:* Antigenic and biological characterization of avian paramyxovirus type 1: Isolates from pigeons, an international collaborative study. Avian Path. 1985, *14*, 365—376.

*Anon.:* Newcastle disease: Outbreaks linked to pigeons. Vet. Rec. 1984, *114*, 305—306.

*Biancifiori, F. & A. Firoroni:* On occurrence of Newcastle disease in pigeons: Virological and serological studies on the isolates. Comp. Immunol. Microbiol. Infect. Dis. 1983, *6*, 247—252.

*Richter, R. & J. Kösters:* Paramyxovirusinfection bei Rassetauben. (Paramyxovirus infection of racing pigeons). Prakt. Tierarzt. 1983, *64*, 250.

*Viaene, N., L. Spanoghe, L. Devriese, B. Bijnens & A. Devos:* Paramyxovirus bij duiven. (Paramyxovirus in pigeons). Vlaams diergeneesk. T. 1983, *52*, 278—286.

SAMMANDRAG

*Paramyxovirus typ 1 (PMV-1) isolerad från sjuka brevduvor. En patogenitetsstudie på kycklingar och unghöns.*

Vid sjukdomsutbrott bland brevduvor i södra Sverige isolerades paramyxovirus av samma typ, som isolerats från sjuka duvor vid utbrott i andra delar av Europa.

En vecka gamla kycklingar infekterade via näsa och öga med ett av dessa PMV-1 isolat insjuknade akut efter 5—11 dagar med symtom lika dem som ses vid neurotrop form av Newcastle sjuka. Även de oympade kycklingarna som gick i samma bur insjuknade med samma symtom en vecka senare. Dödligheten var hög bland kycklingarna.

Unghönor, 120 dagar gamla, som infekterades med samma virus på samma sätt blev däremot inte sjuka och ingen dog.

De döda kycklingarna hade en nonpurulent encephalit och pneumoni, unghönsen endast lindrig encephalit.

PMV-1 återisolerades från alla kycklingar men ej från unghönsen. Både kycklingar och unghönsen bildade antikroppar mot viruset.

*(Received September 13, 1985).*

Reprints may be requested from: Björn Engström, the National Veterinary Institute, Division of Poultry, Box 7073, S-750 07 Uppsala, Sweden.