Virological Studies of Paramyxovirus Type 1 Infection of Pigeons

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Abstract

Over a period of three months in 1985, paramyxovirus type 1 infection was demonstrated for the first time in Canada, in six flocks of pigeons in Ontario, Alberta, and British Columbia. The paramyxovirus type 1 isolates did not cause clinical disease when serially passaged four times in four-to six-week-old chickens, and isolates were classified as lentogenic before and after such serial passage. Further cases of paramyxovirus type 1 clinical disease have not been reported since the last of these six outbreaks in August 1985.

Key words: Paramyxovirus type 1, pigeons.

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Résumé

Études virologiques de l'infection de pigeons par le paramyxovirus du type #1

En 1985, au cours d'une période de trois mois, on a démontré pour la première fois au Canada, l'infection par le myxovirus du type #1 dans six troupeaux de pigeons de l'Ontario, de l'Alberta et de la Colombie-Britannique. Les isolates du virus précité ne causèrent pas de maladie clinique, à la suite de quatre passages successifs chez des poulets âgés de quatre à six semaines; on les classifia comme bradygènes, avant et après ces passages. On n'a pas rapporté d'autres cas cliniques, depuis la dernière de ces six éruptions, en août 1985.

Mots clés: paramyxovirus du type #1, pigeons.

Introduction

Newcastle disease (ND), caused by paramyxovirus type 1 (PMV-1) has been recognized as a disease of pigeons for many years (1, 2), but prior to the early 1980's most reported cases were of a sporadic nature (3, 4, 5). However, a primarily neurotropic form of PMV-1 infection swept through pigeon flocks in many areas of continental Europe in the late 1970's and early 1980's (6, 7). The origin may have been the Middle East (8) where outbreaks of encephalitis of unknown or possibly herpesvirus etiology had been reported in pigeons earlier (9, 10). This epizootic eventually spread to Britain, where it was recognized in racing pigeons in 1983 (11, 12). Initially, the disease was apparently limited to pigeons and no association was made between disease in this species and any outbreaks of disease in domestic poultry (6, 13). However, laboratory studies in Britain indicated that isolates from pigeons could increase in pathogenicity on serial passage in chickens and that, therefore, diseased pigeons could be a source of infection for domestic poultry (13). Such spread subsequently occurred in Britain (14), with serious economic consequences (14, 15, 16).

In 1984, PMV-1 infection was recognized in pigeons in the northeastern USA (17). This report describes the occurrence of the disease in Canada and documents some characteristics of the isolated viruses.

Materials and Methods

Animals and Eggs

Eggs and chickens were obtained from a laboratory flock of isolated non-vaccinated Leghorn-type birds free from hemagglutination-inhibition (HI) antibody to the B1 (18) strain of PMV-1. Clinical data on pigeon flocks were supplied by field veterinarians in reports accompanying submissions of tissues or carcasses.

Virus Isolation and Characterization

Tissue samples were received from a total of six flocks of pigeons in the period June to August 1985 (Table I). Three of the flocks (A, B, C, disease first seen in June) were located in Ontario. The three remaining isolates were from Alberta (D, disease also first seen in June) and British Columbia (E, F, disease seen in August). Viral isolations 1824, 1957, and 2041 (Table I) were made by virologists at Ontario and Alberta Provincial laboratories, and received as infectious allantoic fluid from inoculated chicken embryos.

An approximate 20% (w/v) homogenate of tissues comprising spleen, lung, liver, and brain from affected pigeons was made in 0.05 M phosphate buffered saline (PBS) pH 7.2 and inoculated into 9-11 day embryonated chicken eggs as described previously (13). Two serial passages in eggs were completed before samples were considered negative, with tests for hemagglutination (HA) of allantoic fluid carried out at each passage. Some isolates were made by virologists at provincial laboratories and received as infectious allantoic fluid. These isolates were passaged in eggs once or twice in this manner before use in characterization studies.

All isolates were identified as PMV-1 by the hemagglutination-inhibition (HI) test performed according to standard methods (19, 20), using antiserum prepared against the GB Texas strain. Additional viral characteristics determined were the mean death time (MDT) in ten-day-old embryonated eggs (19), the intravenous pathogenicity index (IVPI) in four-to six-week-old chickens (12), and the intracerebral pathogenicity index (ICPI)

in one-day-old chickens (22). Mean death time values of over 90 h, 70-90 h, and fewer than 70 h, and ICPI values of 0-0.5, 0.5-1.5, and 1-2 were considered to be characteristics of lentogenic, mesogenic, and velogenic isolates, respectively (21, 22).

Intravenous pathogenicity index values for lentogenic strains are zero and for velogenic strains are usually over 2 (maximum value for the IVPI is 3), but mesogenic strains may give values ranging from 0.08 to over 1 (18, 23). The establishment of cut-off points for each level of pathogenicity is not possible.

Passage in Chickens

Four serial passages of viral isolates were completed in four-to six-week-old chickens by intramuscular inoculation (0.5 mL/chicken) according to the method described by Alexander and Parsons (13). In the first passage, infective allantoic fluid was inoculated. Subsequent inocula were prepared from a pool of brian, lung, and spleen collected from each group of five chickens on postinoculation day 5, homogenized as a 10% suspension in PBS pH 7.2, centrifuged to clarify, and filtered through a 0.45 nm Millipore filter. Each serially passaged inoculum was assayed for the presence of PMV-1 virus by inoculation of embryonated eggs.

Characteristics of MDT, ICPI and IVPI were determined before and after passage.

Results

Viral isolation data and classification results for isolates before and after passage in chickens are shown in Table I. Most isolations were made on first passage in eggs, and no difficulty was experienced in demonstrating hemagglutination, or hemagglutination-inhibition with Newcastle disease antiserum. In all cases, MDT and IVPI values for original isolates were within the lentogenic range but ICPI values were within the mesogenic range. Serial passage in chickens did not increase these indices and some values decreased. Isolate F was not recovered after four passages in chickens (Table I). All inoculated chickens remained clinically normal throughout each five-day observation period and no macroscopic abnormality was seen on postmortem examination. Histopathology was not done.

Isolates from flocks A, B, D, and F were examined by Dr. D.J. Alexander, Central Veterinary Laboratory, Weybridge, Surrey, England where, by the indirect immunoperoxidase test, tissue cultures infected by these isolates bound certain monoclonal antibodies in a pattern similar to that of the reference pigeon isolate 561/83 (11) (D.J. Alexander, personal communication). Although ICPI values determined at Weybridge were higher (1.3-1.6) than those determined in our studies (Table I) they were within the mesogenic range, and IVPI values were consistently zero.

The natural disease in affected pigeons was clinically evident as slight dullness, loss of condition and vomiting, to severe incoordination, head-tilting, disorientation in flight, wing and/or leg paralysis, and diarrhea. Mortality rates were not obtained for all flocks but ranged from 3% (flock D) to 40% (flock A).

Discussion

During these outbreaks of PMV-1 infection of pigeons, there was concern that the disease might spread to commercial flocks of poultry. Although outbreaks of PMV-1 infection of pigeons were not associated with concurrent disease in poultry in continental Europe, serious outbreaks of Newcastle disease in commercial poultry in Britian were attributed to PMV-1 strains derived from pigeons (14, 21). These outbreaks, which resulted in the slaughter of about 800,000 fowl at a total control cost of over $4 million (14) clearly demonstrated the potential danger of PMV-1 transmission from pigeons to other avian species. However, it is probable that spread was facilitated by a policy that banned Newcastle disease vaccination of poultry in Britain at that time (24). Subsequently, it has been demonstrated that vaccination with the B1 strain of Newcastle disease virus will protect chickens against challenge with the strain of PMV-1 from pigeons (24).

Serial passage of the isolates, identified in Table I, was performed to examine the potential pathogenicity of these pigeon-derived viruses for chickens. Such studies had enabled British workers to predict correctly the potential pathogenicity for chickens of pigeon PMV-1 strains isolated from pigeons in Britain (13), and had demonstrated that very significant increases in indices such as the IVPI, could occur after as few as two serial

TABLE I
Pigeon PMV-1 Isolation and Classification Results

Flock	Isolate	Date (1985)	MDT	ICPI (Original)[c]	IVPI	MDT	ICPI (After 4 Pass/Chicks)[d]	IVPI
A	1824[a]	June 14	129.9	0.7	0	108.6	0.87	0
	1840	June 20	114.9	0.6	0	93.9	0.3	0
B	1957[a]	June 21	148.4	0.8	0	ND		
	1873	June 22	133	0.71	0	115.5	0.02	0
C	2468	June 27	160.8	0.6	0	127.5	0.7	0
D	2041[b]	June 17	137.1	0.8	0	102.3	0.76	0
E	2225	August 4	107.9	0.59	0	ND		
	3309	August 8	123.7	0.32	0	ND		
F	2226	August 2	142.7	0.6	0	0	0	0

[a]Isolated from pigeon brain tissue by Dr. D. Key, Virologist, Ontario Ministry of Food, Guelph, Ontario
[b]Isolated by Dr. G. Papp-Vid, Virologist, Alberta Dept of Agriculture, Edmonton, Alberta
[c]Original isolate before passage in chickens
[d]Isolated after four serial passages in chickens by intramuscular inoculation at five day intervals
MDT = Mean death time, ICPI = intracerebral pathogenicity index, IVPI = intravenous pathogenicity index (in chickens), ND = Not done

passages. For example, isolates 617/83, 760/83, and 1044/83 increased in their IVPI values from 0, 0.28, and 1.35, to 2.3, 2.03, and 2.13, respectively, after three to four passages (13). However, none of the isolates identified on Table I demonstrated any increase in index values after four serial passages in chickens and none of the inoculated chickens exhibited any evidence of clinical disease. Although these isolates exhibited ICPI values indicative of mesogenic strains (22), they did not show any evidence of ability to cause disease in chickens by routes of inoculation other than intracerebral, and were therefore classed as lentogenic strains. More recently, it has been noted that increased pathogenicity of pigeon isolates on passage in chickens is not a characteristic of all strains of PMV-1 and that in general over the period 1983-85, PMV-1 isolates showed a general decline in pathogenicity for young chickens (27).

Importation of pigeons from the northeastern United States into flocks A, B, and C had occurred in April, May and June, and it is probable that this was the source of infection for these flocks. Contact-infected pigeons may excrete virus for approximately one month (13) and the incubation period can be six weeks or longer (12). Therefore, the period between the disappearance of clinical disease in one flock and appearance of disease in another after carrier introduction could be several weeks. As the virus of Newcastle disease can remain viable in excreted fecal matter for several months (15), the potential for spread of this disease between flocks is significant. Pigeons had been introduced into flocks E and F from flock D just prior to recognition of illness in flock D. Pigeons had been imported into flock D from Holland about three weeks prior to recognition of illness and the first pigeon from which PMV-1 was isolated in flock D was an imported bird which was lame and cachectic. The coincidental onset of disease in both "index" flocks several thousand miles apart (Ontario and Alberta) was apparently due to two different imported sources of infection as there was no known contact between these flocks.

When these outbreaks first occurred there was no PMV-1 vaccine licenced for use in pigeons in Canada. Since it was considered possible that live lentogenic poultry NDV vaccine had been used in pigeons and that a vaccine strain had been the source of these isolations, the isolated viruses were sent to Weybridge for examination (6). Results indicated that these isolates were of pigeon origin.

After the outbreak of PMV-1 infection in flock F (Table I) subsequent cases were not seen, despite investigation of several contact flocks by regulatory veterinarians. At this time, commercially-available killed PMV-1 vaccines are available for use in Canada to provide protection of pigeons against this disease. The cases described in this paper appear to have occurred through outside introduction of carrier birds or contaminated materials into flocks which were not protected by vaccination. In this regard recent work suggests that vaccines for use in pigeons may be preferably prepared from pigeon-origin PMV-1 virus (25), but protection of chickens may be induced by the B1 (chicken origin) strain (24).

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