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INCIDENCE OF PARAMYXOVIRUSES IN FREE-LIVING BIRDS IN 1978—1982

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Summary. — Together 41 paramyxovirus (PMV) strains (25 PMV-1, 10 PMV-4, and 6 PMV-6 serotypes) were isolated from cloacal swabs of 910 free-living birds trapped in West Slovakia from 1978 to 1982. The PMV strains were found in 9, mostly aquatic bird species. Strains belonging to the PMV-1 serotype were isolated yearly, indicating its wide distribution and circulation in nature. The strains of PMV-4 and PMV-6 serotypes found only in 1978—1980, represented the first isolations in Europe. Antigenic analysis by haemagglutination-inhibition (HI), neuraminidase-inhibition (NI), complement-fixation (CF), and gel double diffusion (DD) tests proved the relatedness of the surface antigens of newly isolated PMV strains with those of PMV-4/DuckHong Kong D3/75 and PMV-6/Duck/Hong Kong 311/80 strains. One-way reaction between PMV-4 serotype and mumps virus was demonstrated using hyperimmune rat sera. Electron microscopic observation of isolated virus strains revealed structures typical of PMV.

Key words: *paramyxovirus type 1, type 4, type 6, Newcastle disease virus, aquatic birds*

Introduction

Since 1968 during surveillance of influenza virus in domestic and feral birds, many new viruses belonging to the group of PMV based on their morphology, structure and other properties have been isolated. On the basis of antigenic studies, the isolated virus strains were further divided into 7 serotypes. Of them, PMV-1/NDV and PMV-2/Yucaipa had been known long before 1968, but only the realization of above mentioned surveillance programme made it possible to prove the occurrence of these viruses practically all over the world not only in domestic, but also in wild birds including exotic ones (Lancaster and Alexander, 1975; Alexander *et al.*, 1979; Tůmová *et al.*, 1979; Alexander, 1980; Shortridge *et al.*, 1980). The remaining PMV serotypes were isolated in Southeast Asia, Japan and North America; PMV-3 was also found, after a long interval, in the Netherlands and England.

This paper presents the results of 5-year study on the incidence of PMV in birds in West Slovakia, which besides the finding of PMV-1 serotype resulted in the first isolation from wild birds in Europe of PMV-4 and PMV-6 strains.

Materials and Methods

Material collection and virus isolation. Cloacal swabs were collected in two localities of West Slovakia in 1978—1982 during the autumn hunting seasons (in 1978 and 1979 also from January to March). Collection and isolation methods were described in detail elsewhere (Turek *et al.*, 1982). Isolation experiments were carried out in a laboratory where work with avian para- and orthomyxoviruses is not common.

Serological tests. The HI, NI and DD tests were performed as recommended for influenza diagnosis by standards of the World Health Organization (Palmer *et al.*, 1975). The CF test was carried out with 0.025 ml volumes using 2 units of complement, 3 units of haemolysin and 4% sheep erythrocytes. Antigens for all tests were prepared from allantoic fluids of virus-infected chick embryos. The allantoic fluid for HI test was sonicated in Megason Ultrasonic cleaner; for NI test the virus in the allantoic fluid was concentrated by high-speed centrifugation at 75,000 × g for 2 hr and the sediment was resuspended in phosphate buffered saline. To prepare the antigen for DD test, the virus from the allantoic fluid was purified by adsorption-elution on chick erythrocytes followed by high-speed centrifugation and separation in sucrose (10—60%) gradient, and the purified virus was cleaved with 1% sarcosyl. For CF test, the virus was concentrated by dialysing the allantoic fluid against glycerine.

Virus strains. The prototype strains of PMV-4/Duck/Hong Kong D3/75 and PMV-6/Duck/Hong Kong 334/78 and 199/77 were kindly supplied by Dr. Alexander from the Poultry Department, Weybridge, England.

Sera. Immune sera to the prototype and newly isolated virus strains were prepared in rats, guinea pigs and chickens as described (Tůmová *et al.*, 1979). Hamster serum to mumps virus was kindly provided by Dr. Fedová from the Reference Laboratory for Mumps, Institute of Hygiene and Epidemiology, Prague, Czechoslovakia.

Electron microscopy. A drop of virus suspension was placed on a Formvar carbon coated grid. After 1 min adsorption, the remaining fluid was removed by filtration paper and the preparation was negatively stained by 2% phosphotungstic acid, pH 6.5, for 1 min. Preparations were examined and micrographs prepared in Philips EM 300 microscope.

Results

Virus isolations

Since 1978 systematic investigations on incidence and distribution of influenza viruses have been carried out in free-living, mostly aquatic migrating birds in two localities in West Slovakia (Turek *et al.*, 1983). Cloacal swabs from shot or trapped birds were collected yearly from the end of August to March. The birds were mostly represented by *Anas platyrhynchos*, to a lesser extent by *Anas strepera*, *Aythya ferina* and *Fulica atra* species. Only in 1978 and 1979 the swabs were taken also from other species of wild birds: in autumn from pheasants, in January-March from smaller birds such as raven, pied wagtail and common starling.

The results of the 5-year isolation study are summarized in Table 1. Out of 910 cloacal swabs, 41 strains of PMV were isolated, most frequently from January to March, representing an isolation rate of 4.51%. PMV-1/NDV was a common isolate each year from aquatic and small birds;

Table 1. Isolation of PMV from cloacal swabs of free-living birds trapped in 1978—1982

Year	Virus	Bird species	Number of virus isolations/ birds investigated
1978	PMV-1	*Anas platyrhynchos*	1/99
		Larus ridibundus	1/6
		Sturnus vulgaris	1/29
	PMV-4	*Anas platyrhynchos*	1/99
		Fulica atra	1/14
		Phasianus colchicus	1/62
1979	PMV-1	*Anas platyrhynchos*	4/113
		Aythya ferina	1/13
		Corvus frugilegus	1/1
		Motacilla alba	1/6
		Sturnus vulgaris	2/4
	PMV-4	*Anas platyrhynchos*	7/113
1980	PMV-1	*Anas platyrhynchos*	2/117
		Anas strepera	1/35
	PMV-6	*Anas platyrhynchos*	4/117
		Anas strepera	1/35
		Aythya ferina	1/35
1981	PMV-1	*Anas platyrhynchos*	4/128
		Anas strepera	1/61
		Fulica atra	2/75
1982	PMV-1	*Anas platyrhynchos*	1/20
		Larus ridibundus	2/92
Total			41/910

of the 3.12% isolation rate (25 virus strains isolated out of 799 swabs) the highest proportion (2.38%) was coming from wild ducks.

Six strains of PMV-6 serotype isolated from 187 swabs (3.2% isolation rate), occurred only in 1980. Together 10 PMV-4 strains were isolated over a two-year period, namely in autumn 1978 from wild ducks, coot and pheasant, and from December 1978 to March 1979 from wild ducks only; 7 PMV-4 strains isolated in 1979 accounted for a 6.19% isolation rate. In 1979 the total isolation rate of PMV (11.34%) was the highest found during the whole 5-year study.

Serologic relationships of the surface antigens

Among the isolated virus strains first were determined those of the PMV-1 serotype. For the rest of virus strains the relatedness with influenza A virus was excluded, because they were isolated from swabs of the same collection, which had previously yielded different influenza virus subtypes or their mixtures (Turek *et al.*, 1983). Guinea pig, rat and hen immune sera to the strain *Fulica atra*/Slovakia/78 confirmed in all serological tests the mutual antigenic relatedness of the isolates from 1978 and 1979, and non-relatedness with the PMV-2 and PMV-3 serotypes. Immune sera to the strain *Aythya ferina*/Slovakia/80 revealed in corresponding serological tests a further distinct type of PMV, embracing all virus strains isolated in 1980.

Table 2. Antigenic relatedness of isolated PMV based on the results of HI and NI tests

Sera to given viruses		Animal species	Antigens									
			1	2	3	4	5	6	7	8	9	10
1	PMV-1	rat	160*	neg.	neg.	neg.	n.t.	n.t.	n.t.	neg.	n.t.	n.t.
	NDV	rat	(40)	neg.	neg.	neg.	neg.	n.t.	n.t.	neg.	n.t.	n.t.
2	PMV-2	guinea pig	neg.	160	neg.	neg.	neg.	n.t.	n.t.	neg.	n.t.	n.t.
	Yucaipa	guinea pig	neg.	(1500)	neg.	neg.	neg.	n.t.	n.t.	neg.	n.t.	n.t.
3	PMV-3	rat	10	neg.	160	10	10	n.t.	n.t.	neg.	n.t.	n.t.
	Turkey/Wis/68	guinea pig	neg.	neg.	(330)	neg.	neg.	n.t.	n.t.	neg.	n.t.	n.t.
4	PMV-4	guinea pig	neg.	neg.	neg.	160	80	80	320	neg.	n.t.	n.t.
	DK/Hong Kong/75	guinea pig	neg.	neg.	neg.	(1280)	(140)	(1280)	(1280)	neg.	n.t.	n.t.
5		guinea pig	neg.	neg.	neg.	40	160	80	80	neg.	n.t.	n.t.
	Fulica atra/75	rat		n.t.	n.t.	(200)	(1100)	(700)	(600)	neg.	n.t.	n.t.
6		guinea pig	n.t.	n.t.	n.t.	80	40	160	160	neg.	n.t.	n.t.
	Anas plat. III/78	guinea pig	n.t.	n.t.	n.t.	n.t.	n.t.	(1200)	(1200)	n.t.	n.t.	n.t.
7	PMV-6	guinea pig	n.t.	n.t.	n.t.	80	40	80	160	n.t.	n.t.	n.t.
	Phasianus colch./78	guinea pig	n.t.	n.t.	n.t.	n.t.	n.t.	(1280)	(1280)	n.t.	n.t.	n.t.
8		guinea pig	neg.	neg.	neg.	neg.	neg.	n.t.	n.t.	320	160	160
	Duck/Hong Kong 334/78	guinea pig	neg.	neg.	neg.	neg.	neg.	n.t.	n.t.	(1280)	(1000)	n.t.
9		guinea pig	neg.	neg.	neg.	neg.	neg.	n.t.	n.t.	80	320	160
	Aythya ferina/80	guinea pig	neg.	neg.	neg.	neg.	neg.	n.t.	n.t.	(1280)	(1280)	n.t.
10		guinea pig	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	80	160	80
	Anas plat. 311/80	guinea pig	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	(400)	(640)	n.t.

* Titres of HI and NI (in parenthesis) antibodies in the sera to given virus strains; neg. — negative; n.t. — not tested

Table 3. Antigenic relationships between PMV-4 strains and Enders strain of mumps virus

	Sera to virus strains	Animal species	Titres of CF and HI antibodies in sera to given virus strains					
			CF			HI		
			1	2	3	1	2	3
1	PMV-4 *Fulica atra*/78	rat	320	160	40	160	80	20
		guinea pig	320	80	neg.	n.t.	n.t.	n.t.
2	PMV-4 *Anas plat.* III/78	rat	1280	1280	80	80	80	20
		guinea pig	320	320	neg.	n.t.	n.t.	n.t.
	PMV-4 *Phas. colch.*/78	rat	320	640	20	160	160	40
		guinea pig	160	160	neg.	n.t.	n.t.	n.t.
3	Parotitis (Enders)	hamster	neg.	neg.	640	neg.	neg.	1280

neg. — negative; n.t. — not tested.

Relatedness of the surface antigens of selected newly isolated virus strains with those of prototype strains isolated in Hong Kong is given in Table 2, presenting the results of HI and NI tests, and in Figs 1 and 2, presenting the results of DD test. Cross-reactions between strains under study clearly confirmed that isolates from 1978 and 1979 belonged to type PMV-4 and isolates from 1980 to type PMV-6, respectively. The results obtained demonstrated close antigenic relatedness of haemagglutinin and neuraminidase of our isolates with those of Southeast Asia.

In HI test mostly were employed immune guinea pig sera, which are known to give very specific reactions between PMV strains of the same serotype. In NI and DD tests, any immune serum, rat including, with a sufficiently high antibody titre can be used. By means of hyperimmune rat sera, the heterotype serological reactions known for some types of PMV could be confirmed in HI test. An apparent one-way reaction of PMV-3 antiserum with NDV and PMV-4 is shown in Table 2. Similar one-way reactions between sera to PMV-4 serotype and mumps virus were found in HI and CF tests (Table 3) and in DD test (Fig. 3). For DD test, however, the sera had to be concentrated by diluting the lyophilized serum samples to 1/3 of the original volume to obtain sufficiently distinct precipitation lines. Such reactions were not observed in NI test, even if other strains of human paramyxoviruses were used. More detailed elucidation of these relationships, their cause and importance, is the subject of further study.

Biological and morphological characterization of newly isolated virus strains

Biological properties of newly isolated virus strains correspond to those of avian PMV. They easily and rapidly replicate in chick embryos, possess haemolytic activity and they are insensitive to non-specific inhibitors. Strains of both PMV-4 and PMV-6 serotypes are weak antigens as confirmed not

only by difficulties of preparing the specific immune sera, but also by determining low antibody levels in sera of wild birds.

Morphology of the newly isolated virus strains is identical with that of other PMV (Figs 4 and 5). Virions are pleomorphic, have dense peripheral structures so that their individual surface projections are not distinct. After disruption of the virus coat, the inner nucleocapsid is released, resulting in a clearly discernible helix of ribonucleoprotein (Figs 4 and 5).

Discussion

Isolation from wild birds of 3 types of PMV, especially of PMV-4 and PMV-6 which are the first isolates in Europe and among the first ones over the world, has given further evidence to the fact that birds are obviously more frequent reservoirs of viral and bacterial agents than it was previously supposed (Janout *et al.*, 1979). The most common is probably the PMV-1/NDV serotype known since 1927 to cause manifest disease in poultry with a wide spectrum of symptoms. At present, however, it can be found frequently all over the world in many bird species, free-living birds including, causing mostly asymptomatic infection, often mixed with other PMV or with influenza virus (Lancaster and Alexander, 1975; Shortridge and Alexander, 1978).

In our 5-year study, the PMV-1 was isolated yearly from the most diffferent species of aquatic and small birds, namely from ducks (more than a half of all isolates of PMV-1, i.e. 15 of 25, were from ducks). The isolation rate from *Anas platyrhyanchos* varied in individual years from 1.97 to 2.67%, except in 1979, when 9 PMV-1 strains were isolated from 137 swabs representing the highest isolation rate of 6.5%. An accidental isolation of PMV-1 from *Anas platyrhynchos* in England was described also by Alexander *et al.* (1979); it seems, however, that ubiquity of PMV-1 as to localities and bird species had to be proved by further systematic studies.

Strains of PMV-4 and PMV-6 were isolated in West Slovakia 3 years after the first isolations of these serotypes had been reported from wild ducks in the U.S.A. (Alexander *et al.*, 1979) and from domestic ducks in Hong Kong (Shortridge *et al.*, 1980). Antigenic relatedness of these PMV strains and the interval between their isolations admit the possibility of their transmission by migrating birds to Europe from Southeast Asia. If so, it is most probable that these serotypes of PMV will be sooner or later detected also in other European countries; surveillance in poultry-farming facilities and checking for viruses in wild birds could contribute to the elucidation of this problem. Of course, a hypothesis that these and probably some other serotypes of PMV might be wides pread in the duck population in the form of inapparent infections (Alexander *et al.*, 1979), cannot be also excluded. PMV mixed with influenza virus had been present in the duck population already for a relatively long time, in the remote past escaping our attention because as a common practice in isolations of influenza virus strains from birds only possible contamination by NDV had been taken into account.

Isolation of PMV-4 from pheasant indicates that similarly as in other PMV serotypes, aquatic birds are not exclusive hosts in which this type of PMV can be detected. As a source of infection may serve vegetation contaminated by excrements in stopovers of wild duck migration. In this way, the virus can be spread and infect further birds, including poultry.

As to morphology and biological properties, PMV seem to form comparatively homogeneous group of viruses. This has been confirmed also by our present results of morphological, biological and serological studies with newly isolated PMV strains. Differences in antibody titres in serological cross-reactions can be attributed rather to different avidity of strains than to actual antigenic differences in the sense of antigenic drift. This type of gradual and permanent changes is infrequent in PMV, because of the character of their genome. Though variants of the same serotype were described (Alexander, 1980), their frequency, cause and importance are not precisely known. Their existence cannot be unambiguously explained even by demonstration of structural differences between PMV strains as attempted by Alexander and Collins (1981) using the polyacrylamide gel electrophoresis. However, they determined the basic features of relatedness of structural polypeptides in PMV strains of the same type and differences between strains of different serotypes.

The nature of heterotype reactions has not been elucidated yet. However, the results of Alexander and Collins (1981) suggest some degree of identity in the profiles of polypeptides and in their migration, respectively, in the case of strains of PMV-1, PMV-3 and further serotypes, in which these reactions had been proved. In this direction, the results of our study on the antigenic relationships between PMV-4 serotype and mumps virus will be the subject of our further communication.

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Explanation to Figures (Plates XVIII—XX):

Fig. 1. Results of DD test with the PMV-4/*Fulica atra*/78 isolate.
In the middle: Antigen of PMV-4/*Fulica atra*/78 strain cleaved by sarcosyl.
On the circumference: sera to virus strains (in parentheses animal species in which the sera were prepared) as follows: 1. *Fulica atra*/78 (rat); 2. *Anas platyrhynchos* III/78 (guinea pig); 3. *Phasianus colchicus*/78 (guinea pig); 4. *Anas platyrhynchos* 19/78 (guinea pig); 5. influenza A/RNP (guinea pig); 6. PMV-1/NDV (rat); 7. PMV-2/Yucaipa (rat); 8. PMV-3/Turkey/Wisconsin/68 (rat); 9. PMV-4/Duck/Hong Kong/75 (hen); 10. PMV-6/Duck/Hong Kong 199/77 (guinea pig).

Fig. 2. Results of DD test with the PMV-6/*Aythya ferina*/80 isolate.
In the middle: Antigen of PMV-6/*Aythya ferina*/80 strain cleaved by sarcosyl.
On the circumference: sera to virus strains (in parentheses animal species in which the sera were prepared) as follows: 1. PMV-6/Duck/Hong Kong 334/78 (guinea pig); 2. PMV-6/*Aythya ferina*/80 (guinea pig); 3. PMV-6/*Anas platyrhynchos* 311/80 (guinea pig); 4. PMV-6/Duck/Hong Kong 199/77 (guinea pig); 5.—10. The same sera as given in Fig. 1.

Fig. 3. One-way reaction between sera to PMV-4 serotype and mumps virus.
In the middle: Virus antigens as follows: A. PMV-4/Duck/Hong Kong D3/75; B. Mumps (Enders strain); C. PMV-4/*Fulica atra*/Slovakia/78.
On the circumstance: sera to virus strain (lyophilized sera diluted to the 1/3 of original volume; in parentheses animal species in which the sera were prepared: 1. Enders mumps virus (hamster); 2. *Fulica atra*/Slovakia/78 (rat); 3. Duck/Hong Kong D3/75 (rat).

Fig. 4. Negative staining of PMV-4/*Anas platyrhynchos*/Slovakia/III/78 isolate with phosphotungstic acid.
Viral particles with inner filaments of nucleoprotein are seen. Spikes are preserved on the surface of viral particles. Distinct small protrusions are visible in the vicinity of a damaged viral particle. × 160,000.

Fig. 5. Negative staining of PMV-6/*Anas platyrhynchos*/Slovakia/311/80 isolate with phosphotungstic acid.
Disrupted negatively stained viral particle with a diameter of 360 nm. Inner filaments (nucleoprotein) with an average diameter of 18 nm projecting from the virion. Spikes on the virion surface are well-preserved. × 160,000.