

STUDIES UPON THE BASIC CHARACTERISTICS OF AVIAN PARAMYXOVIRUS TYPE 1 STRAINS, ISOLATED FROM PIGEONS IN BULGARIA

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Summary

Hadjiev, G., 2002. Studies upon the basic characteristics of avian paramyxovirus type 1 strains, isolated from pigeons in Bulgaria. *Bulg. J. Vet. Med.*, **5**, No 1, 1 – 18.

The basic characteristics of 3 avian paramyxovirus type I (A/PMV-1) strains, isolated from domestic pigeons in Bulgaria, were parallelly studied with reference strains of the Newcastle disease virus (NDV) belonging to all pathotypes. According to generally accepted standard biological tests, the strains from pigeons referred to the mesogenic pathotype of NDV. According to their plaque forming ability in primary monolayer cell cultures of chicken embryo fibroblasts (CC/CEF) under agar cover, the local strains from pigeons behaved like velogenic NDV strains. Pigeon strains showed a high agglutination activity against chick erythrocytes and their haemagglutinins were stable at 56°C. NDV-sensitive chickens, aged 5-8 weeks, inoculated intramuscularly, intravenously, intracloacally or intra-conjunctivally with local A/PMV-1 strains, obtained from pigeons as well as non-infected chickens from the same batch, put in contact with infected ones, developed an occult infection. Both inoculated and contact chickens underwent an immunological transformation – they reacted with formation of high titres of specific serum antibodies that protected them from disease or death after challenge with a definitely lethal dose of a velogenic-viscerotropic NDV strain. Our previous studies upon Bulgarian A/PMV-1 strains isolated from pigeons did not suggest that they were really dangerous for poultry health by respect to Newcastle disease. This statement is supported by other observations in the country as well.

Key words: Newcastle disease, paramyxovirus type 1, pigeon

INTRODUCTION

In 1982, an infectious disease on domestic pigeons (*Columbia livia*¹) that acquired a panzootic character of incidence appeared in Europe (including Bulgaria) (Hadjiev, 1988). Clinically it was similar to the velogenic-neurotropic form of the Newcastle disease (ND) (Vindevogel et al., 1982; Richter et al., 1983; Viane et al., 1983;

Anonymous, 1984c; Engström et al., 1985; Tangredi, 1985; Hadjiev, 1984; 1986; Collins et al., 1989). It is ascertained that the etiological agent belongs to the avian paramyxovirus serotype-1 (A/PMV-1), whose principal representative is the Newcastle disease virus (NDV) (Vindevogel et al., 1982; Fleury et al., 1983; Alexander et al., 1984a; Anonymous, 1984b, 1984c; Eisa and Omer, 1984; Guittet et al., 1984; Sicole et al., 1984; Teremi, 1984; Alexander et al., 1985; Kaleta et al., 1985; Tangredi, 1985;

¹ The *Columbia livia* species, class *Columbiformes*, includes about 800 varieties: racing (carrier) pigeons, decorative pigeons and meat breeds (Jakoby et al., 1983).

Vasichkek, 1985; Saghy et al., 1985; Singh, 1991).

Despite the spontaneous cases of the disease (Hanson and Sinha, 1952; Richter and Goosens, 1971; Stewart, 1971; Richter et al., 1983; Eisa and Omer, 1984), pigeons are relatively resistant to a natural NDV infection (Ahmed and Reda, 1967).

Kaleta et al. (1985) have confirmed that the BVC-78 virus, isolated in the Middle East (Iraq), believed to cause the viral encephalomyelitis in pigeons, was a typical representative of A/PMV-1, that was initially spread in Mesopotamia, in the Nile's delta and Sudan and was transmitted by expositions, competitions and trade in Italy as well. This virus is, in fact, the first of known A/PMV-1 isolates, responsible for the panzootic among pigeons after 1982 in Europe and other regions of the world. According to another theory (Anonymous, 1984b), the panzootic began in 1980 in Egypt and then involved whole Europe, while a third theory (Anonymous, 1984c) speculates that the infection has originated from Sudan, has penetrated in Egypt and via the Mediterranean countries – was spread in Europe and all around the world.

The agent of paramyxovirus type 1 could be isolated from the viscera of recently dead and/or acutely sick pigeons. It could be serologically identified via the haemagglutination inhibition (HI) test, used also for retrospective (sero)-diagnostics (Biancifiori and Fiorooni, 1983; Viaene et al., 1983; Anonymous, 1984a; Eisa and Omer, 1984; Engström et al., 1985; Vasichkek, 1985).

The virulence of pigeon A/PMV-1 isolates is various. It is assessed through routine methods for NDV strains (OIE Manual, 1966; Alexander, 1998).

In a comparative study with 9 monoclonal antibodies against the NDV, Ulster

2Cq strain, Alexander et al. (1985b) reported that the binding of 53 viral isolates from pigeons, originating from 12 countries, was identical, i.e. they all belonged to the A/PMV-1 serotype and were antigenically identical. Pigeon isolates however, showed some difference in the binding of those antibodies compared to the authentic NDV strains.

It was found out (Hadjiev, 1988; 2000) that the antigenic coincidence among various pigeon A/PMV-1 strains based on haemagglutinin was bigger in quantitative terms than that among pigeon strains and reference NDV strains.

The strains from the A/PMV-1 group, adapted to pigeons, are still subject of various studies because of some biological features. The question whether and to what extent the characteristics of variant A/PMV-1 strains, particularly those, causing the panzootic after 1982, belong to a single pathotype like the authentic NDV strains, is however not fully explicated. Our comparative studies on the characterization of 3 A/PMV-1 strains, isolated from pigeons in Bulgaria, are dedicated to this problem too.

MATERIALS AND METHODS

Material

Viruses. The local A/PMV-1 strains, named Pigeon/Vd.Bg/64/1984, Pigeon/Pd.Bg/78/1984 and Pigeon/Sf.Bg/80/1984 respectively, were isolated in chick embryos from dead and/or ill pigeons, representatives of cultivated breeds from various regions in Bulgaria. Before dying, the pigeons manifested severe neurological signs, including torticollis.

For comparison of results, we used reference NDV strains: Hitchner-B₁ and La Sota – lentogenic; Roakin, Komarov and Hertfordshire [H] – mesogenic; Te-

xas-GB – velogenic-neurotropic and the local chicken strain Haskovo – velogenic-viscerotropic (Hadjiev, 1973; 1988).

Chicken embryos (CE). They were obtained from Leghorn poultry eggs ($n \approx 4000$) (Hybrid Centre of Poultry Breeding, Kostinbrod), free from acute infectious diseases, vaccinated against ND at least 2 months before.

Chickens. According to the objective of the experiments, 1-day-old chickens ($n \approx 250$) were used for determination of the intracerebral pathogenicity index (ICPI) and 5-8-weeks-old birds ($n \approx 300$) – for determination of the intravenous pathogenicity index (IVPI), mean death time by intracloacal inoculation pathogenicity test (MDT/CIPT), for contact controls and control infections (challenges).

Pigeons. Clinically healthy, well developed and active carrier pigeons aged 6-12 months, serologically negative against NDV Hitchner B1 and La Sota strains and against one of the local A/PMV-1 strains by the HI test, were used ($n \approx 60$).

Cell cultures. They were chicken embryo fibroblasts (CEF) and were obtained by fractional trypsinization. CEF were supplied as suspension from the Specialized Laboratory of Tissue Cultures, National Diagnostic and Research Veterinary Medical Institute, Sofia, Bulgaria.

Chicken erythrocytes. Blood for obtaining erythrocytes was collected from hens – permanent donors and processed by routine methods. As anticoagulant, 4% sodium citrate or Aksever's solution were used (Hadjiev, 1988).

Sera. Positive sera against the authentic NDV strains and against A/PMV-1 strains from pigeons were obtained from hens, hyperimmunized according to a schedule (Hadjiev, 1973). Convalescent sera from pigeons, affected by A/PMV-1,

were also used.

Methods

Virus isolation. It was performed in 9–11-days old CE. As source material, brain, trachea, lungs and other visceral suspensions, including parts of the gut were used (combined samples from dead or acutely sick pigeons, euthanized with a diagnostic purpose). When the result was negative, 2–3 additional blind passages were performed and sometimes, a new attempt with source suspensions.

Virus identification. It was done serologically, using a known, NDV-positive chicken serum in the HI test – beta procedure with 8 haemagglutination units (HAU), macro- and microvariants (Hadjiev, 1988).

Mean embryo infective dose (EID_{50}) and *mean embryo lethal dose (ELD_{50})*. They were determined in 10-days-old CE according to Reed and Muench (1938)².

Mean death time of the minimum lethal dose (MDT/MLD). The parameter was determined in 10-day-old CE according to the method of Hanson and Brandly (1955)³.

Intracerebral pathogenicity index (ICPI). It was determined in 1-day-old chickens ($n=10$), inoculated intracerebrally with 0.1 ml undiluted allantoic fluid containing the virus (Hanson, 1980)⁴.

Intravenous pathogenicity index

to the virulence, respectively the lethality of strains for CE.

³ MDT/MLD values correlate negatively with strain pathogenicity and has been used to classify virus strains into velogenic (taking under 60 hours to kill); mesogenic (taking between 60 and 90 hours to kill); and lentogenic (taking more than 90 hours to kill).

⁴ ICPI values range from 0.00 to 2.00 and are proportionate to the neuropathogenicity of strains.

(IVPI). It was determined in 6-8-week-old chickens (n=8) (Rosenwald et al., 1959)⁵.

Mean death time by intracloacal pathogenicity (MDT/ICP) test. It was performed on 6-week-old chickens as a qualitative and quantitative parameter of pathogenicity using the method of McDaniel and Orsborn (1973) for infection of chickens combined with the method of Alexander and Allan (1974) for calculation of the mean death time⁶

Plaque-forming capacity (PFC). It was studied on primary cell cultures from chicken embryo fibroblasts (CC/CEF) at 37 °C under conditions of hermeticity. After the inoculation of the CC/CEF monolayer, it was overlaid by 1.1% agar (Noble, Difco) added in a maintenance nutrient medium (with 2.5% newborn calf serum, without phenol red, pH 7.4). The developing (second or colour) agar layer, without serum and phenol red, but with neutral red, final dilution of 1:10000 was put at hour 72. The results were determined on the following day. The colour, shape and number of plaques was determined visually while the size and margins of plaques – with an invert microscope with micrometer eyepiece. The plaques were ranked as miniature (diameter <1 mm); small (1.1–1.5 mm); medium (1.6–2.5 mm) and large (>2.5 mm) (Daniel and Hanson, 1968). Plaque titres were calculated in plaque forming units/log10/ml (Villegas and Purchase, 1980).

proportionate to the pathogenicity of avian strains.

⁶ MDT/ICP scores range from 0.00 to 10.00 and are inversely proportionate to the pathogenicity of avian strains. This test could differentiate lento- and mesogenic from velogenic NDV strains, from one part and velogenic-viscerotropic from velogenic-neurotropic strains, on the other.

Elution test. The type of chicken erythrocytes elution following adsorption upon CC/CEF, infected with tested viruses, was determined according to Beard et al. (1970).

Thermostability of haemagglutinins. It was studied at 56°C according to the original method of Hanson et al. (1949)⁷.

RESULTS

The results from the investigations on A/PMV-1 strains, isolated from pigeons in Bulgaria and the reference NDV strains, used for comparison, are presented in Table 1.

ELD₅₀ values for pigeon strains and those for lentogenic reference Hitchner B₁ and La Sota strains were lower than the respective EID₅₀ values as result of the presence of living CE up to the end of the period of observation (7 days), although they were proven to be infected (Fig. 1).

MDT/MLD values for 10-day-old CE of pigeon A/PMV-1 strains ranged between 75.2 to 88.0 h, characteristic for classic mesogenic NDV strains. MLD values for two of pigeon strains were lower compared to parallelly studied meso- and velogenic reference strains (Fig. 2).

ICPI values in 1-day-old chickens of pigeon A/PMV-1 strains were between 0.37–0.56, i.e. lower than those of velogenic and mesogenic reference NDV strains (Fig. 3). The incubation period of the diseases varied from 3 to 8 days in experimental chickens while the death occurred not before 4-5 days following inoculation with pigeon strains and not in all birds included in the experiment.

Chickens, used for IVPI determination, showed no signs of disease after in-

⁷ are proportionate to pathogenicity of NDV strains.

Table 1. General characteristics of three A/PMV-1 strains, isolated from pigeons in Bulgaria compared to reference NDV strains isolated from chickens.

PARAMETERS	A/PMV-1 STRAINS									
	Pigeon/Vd. Bg/64/1984	Pigeon/Pd. Bg/78/1984	Pigeon/Sf. Bg/80/1984	NDV - Hitchner B ₁	NDV - La Sota	NDV - Roakin	NDV - Komarov	NDV-Hert- fordshire (H)	NDV - Texas GB	NDV - Haskovo
ELD ₅₀ (log ₁₀ /ml)	6.84	8.11	6.14	3.00	4.50	10.07	9.37	8.68	10.10	9.57
EID ₅₀ (log ₁₀ /ml)	6.99	8.61	6.96	8.59	9.18	10.07	9.37	8.68	10.10	9.57
MDT/MLD (h)	79.5	75.2	88.0	∞	∞	73.0	72.0	60.8	44.0	54.4
MLD (at the virus dilution)	10 ⁻⁶	10 ⁻¹	10 ⁻³	-	-	10 ⁻⁸	10 ⁻⁶	10 ⁻⁶	10 ⁻⁸	10 ⁻⁸
ICPI	0.38	0.37	0.56	0.00	0.00	0.45	0.64	0.79	1.55	1.80
IVPI	0.00	0.00	0.00	0.00	0.00	0.05	0.18	0.00	2.25	2.23
MDT/ICP	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	4.8	4.3
CC/CEF (log ₁₀ /ml)	7.22	9.14	9.32	0.00	0.00	7.87	7.86	6.92	7.95	8.41
HA titres (reciprocal values)	1280	1280	640	5120	2560	1280	1280	1280	1280	1280
Thermostability of haemagglutinins at 56 °C (min)	60	120	60	< 15	< 15	< 15	< 15	15	60	30

ELD₅₀ = mean embryo lethal dose; EID₅₀ = mean embryo infective dose; MDT = mean death time; MLD = minimal lethal dose; MDT/MLD = mean death time of the minimal lethal dose; ICPI = intracerebral pathogenicity index; IVPI = intravenous pathogenicity index; MDT/ICP = mean death time by intracloacal pathogenicity test; CC/CEF = cell culture from chicken embryo fibroblast (cells); HA = haemagglutination; ∞ = MDT is endless because the MLD is unestablishable; — = MLD is indefinable.

fection with pigeon A/PMV-1 strains, but underwent an immunological transformation. The mean geometric titres ($\bar{G}$) of specific haemagglutinins in blood sera reached 1:144.7 to 1:246.7 at day 17 from the beginning of the experiment in the HI

test – macrovariant and NDV Hitchner B₁ antigen. The same sera, included in a HI test with homologous (pigeon) antigens, showed a considerably increased avidity of antibodies – titres ranging from 1:493.5 to 1:761.1.

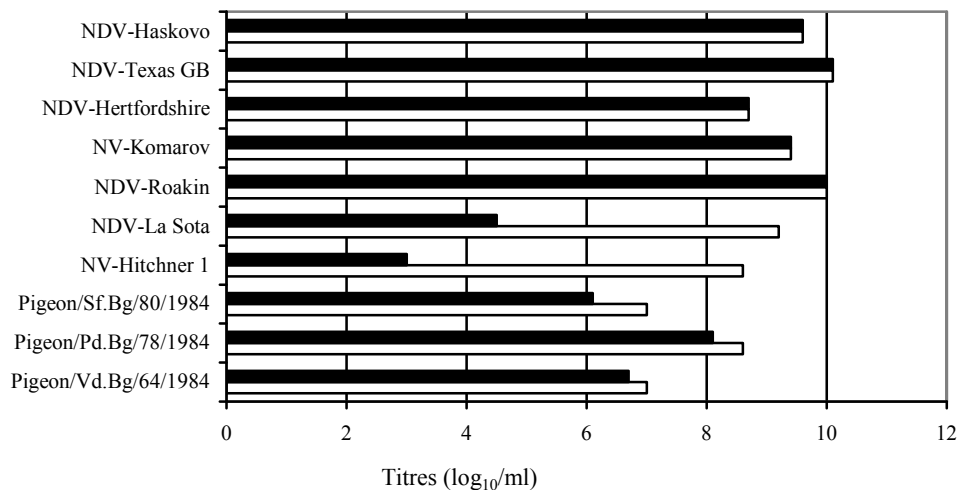


Fig. 1. Mean embryo infective dose (EID₅₀;) and mean embryo lethal dose (ELD₅₀;) (titres) of three Bulgarian A/PMV-1 strains from pigeons and standard NDV strains in chicken embryos.

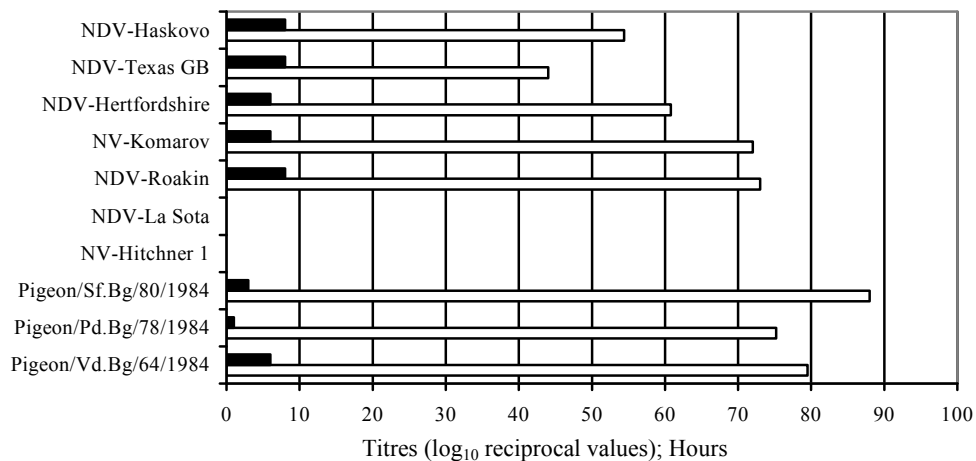


Fig. 2. Minimal lethal dose (titre) (MLD;) and mean death time (h) from the MLD (MDT/MLD;) of three Bulgarian A/PMV-1 strains from pigeons and reference NDV strains in 10-days old chicken embryos.

The differences between titres with heterologous and homologous antigens were statistically significant ($p < 0.01$).

IVPI values in chickens for pigeon viruses were zero (Fig. 3); such values were characteristic for chicken NDV lentogenic and most of mesogenic strains.

NDV-sensitive chickens (serologically negative) at the age of 6 weeks (controls) that were put in a close contact with experimental chickens from the first day of the study in order to determine IVPI, showed no signs of disease or lethality up to day 17 (the end of observations) and underwent an immunological transformation with formation of serum antihaemagglutinins in positive titres. Both control and experimental chickens survived the challenge with a 100% lethal dose of the velogenic-viscerotropic Haskovo strain, performed 17 days after the infection. They showed no signs of diseases or lethality 15 days after the challenge (observation period) but reacted with sharp in-

crease of serum antihaemagglutinins.

MDT/ICP test values of tested local pigeon strains and of lentogenic and mesogenic reference strains in chickens were assessed to be 10 days or more than 10 days, i.e. tested chickens ($n=10$ in an experimental group) survived during the 10-day period of observation (Fig. 4). Experimental chickens challenged with local pigeon A/PMV-1 strains demonstrated an ascendent seroconversion of antihaemagglutinins and became fully resistant to challenge with a 100% lethal dose of the velogenic-viscerotropic Haskovo strain without any clinical signs or lethality.

A part of carrier pigeons aged between 6 months and 1 year, put in a continuous direct contact with experimental chickens, showed the characteristic clinical signs and died within day 52th (the end of observation period). The necropsy and the macroscopic findings in dead pigeons were negative. The pigeons that survived formed specific serum antihaemaggluti-

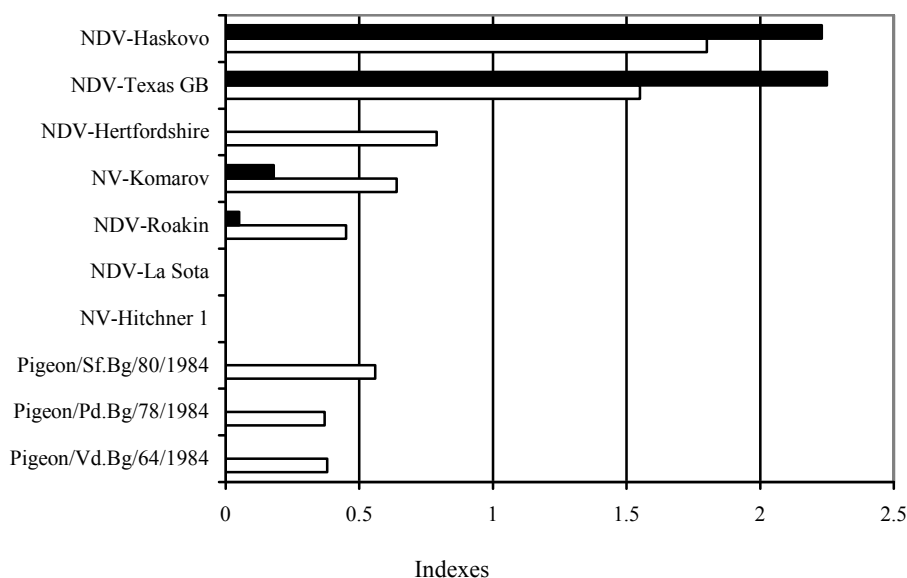


Fig. 3. Intracerebral pathogenicity index (ICPI;) and intravenous pathogenicity index (IVPI;) of three Bulgarian A/PMV-1 strains from pigeons and reference NDV strains.

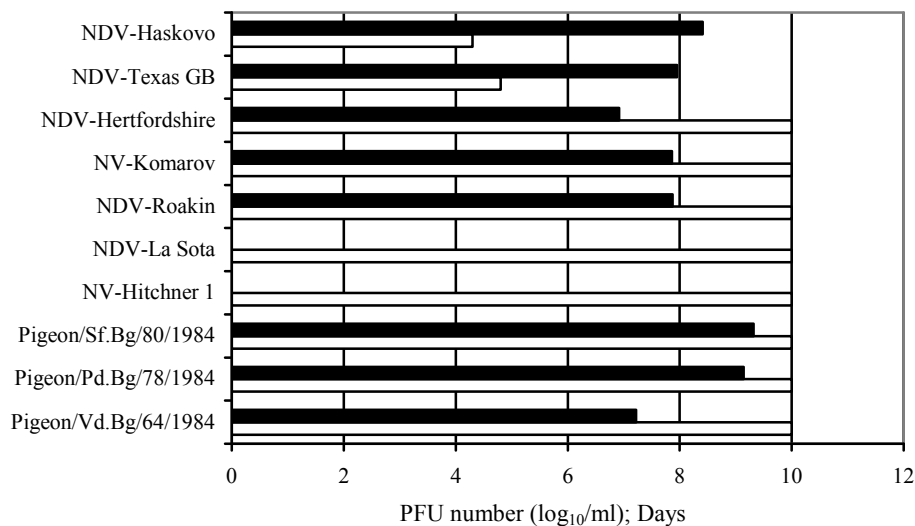


Fig. 4. Mean death time (days) by intracloacal inoculation pathogenicity (MDT/ICP;) test in chickens and plaque-forming units (PFU) into cell cultures from chicken embryo fibroblasts (CC/CEF;) of three Bulgarian A/PMV-1 strains from pigeons and reference NDV strains.

Table 2. Plaque populations of studied viral strains according to the size of plaques

Strains	Size of plaques (diameter in mm)				Patho-type ¹
	Miniature (less than 1.0 mm), %	Small (1.1–1.5 mm), %	Medium (1.6–2.5 mm), %	Large (over 2.5 mm), %	
Pigeon/Vd.Bg/64/1984	13	29	50	8	VG
Pigeon/Pd.Bg/64/1984	56	31	13	0	VG
Pigeon/Sf. Bg/80/1984	36	31	33	0	VG
NDV – Hitchner B ₁	0	0	0	0	LG
NDV – La Sota	0	0	0	0	LG
NDV – Roakin	100	0	0	0	MG
NDV – Komarov	100	0	0	0	MG
NDV – Hertfordshire (H)	100	0	0	0	MG
NDV – Texas GB	69	20	11	0	VG
NDV – Haskovo	35	15	48	2	VG

¹ According to the character of the plaque populations: VG = velogenic; LG = lentogenic; MG = mesogenic.

nins with titres up to 1:160.

The local pigeon A/PMV-1 strains and reference NDV strains, used in our experiments, with the exception of lento-

genic reference Hitchner B₁ and La Sota strains were very well cultivated in primary monolayer CC/CEF. They formed light (dull white) plaques with round or

oval shape. Data for plaque structure according to the size of formed plaques and their affiliation to a given viral pathotype are given in Table 2 and Fig. 5.

The reference mesogenic NDV strains, included in our experiment (Roakin, Komarov, H) showed plaque populations composed by tiny plaques (less than 1.0 mm in diameter). Plaque populations of velogenic reference NDV strains (Texas-GB and Haskovo) as well as the local pigeon A/PMV-1 strains exhibited a diversity of tiny, small, medium and sometimes large plaques, i.e. by respect to plaque size, they produced heterogenous populations. Large plaques (bigger than 2.5 mm) were produced only by the Pigeon/Vd.Bg/64/1984 strain and the velogenic-

viscerotropic Haskovo NDV strain, but their percentages in the population were relatively low (8% and 2%, respectively). The plaque populations of local pigeon A/PMV-1 strains were predominantly formed by tiny (less than 1.0 mm) plaques (about 35%), followed by medium (between 1.6 and 2.5 mm) with 32% and small plaques (between 1.1 and 1.5 mm) with 30%. Under the same conditions, lentogenic NDV strains did not form plaques. The plaque-forming units for the various strains are presented on Fig. 4.

The margins of plaques, assessed under microscope, were not smooth and regular, but less or more irregular, without any connection with strain features (Figs. 5, 6).

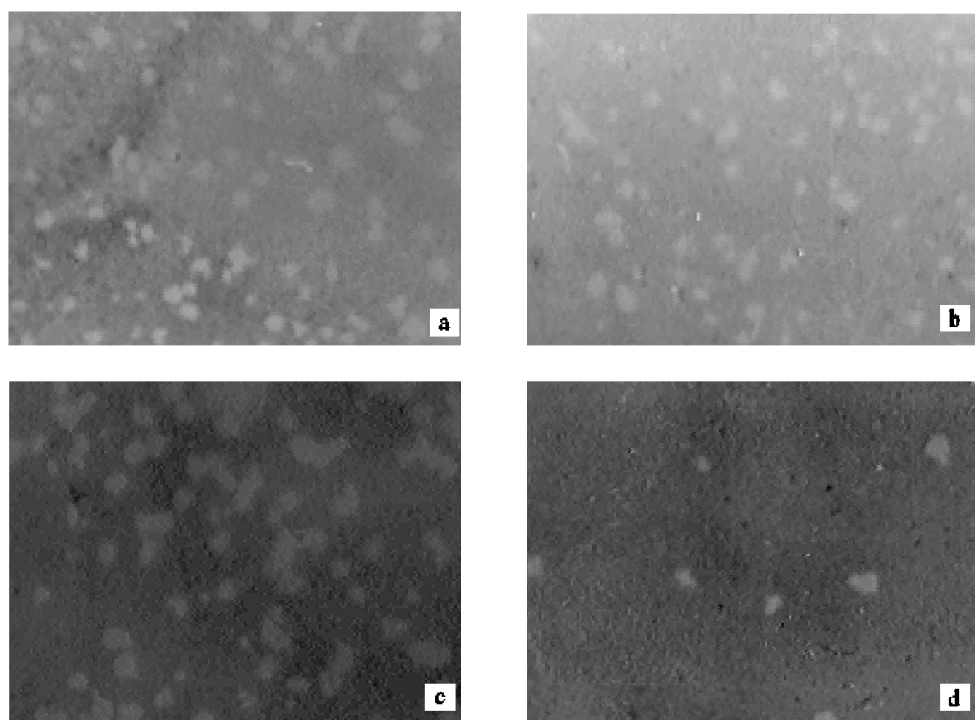


Fig. 5. Heterogeneity of pigeon A/PMV-1 plaque populations in CC/CEF under agar formed by the following strains: a. Pigeon/Vd.Bg/64/1984 – 10^{-4} (2×); b. Pigeon/Sf.Bg/80/1984 – 10^{-4} (2×); c. Pigeon/Pd.Bg/78/1984 – 10^{-5} (2×); d. Pigeon/Pd.Bg/78/1984 – 10^{-6} (2×).

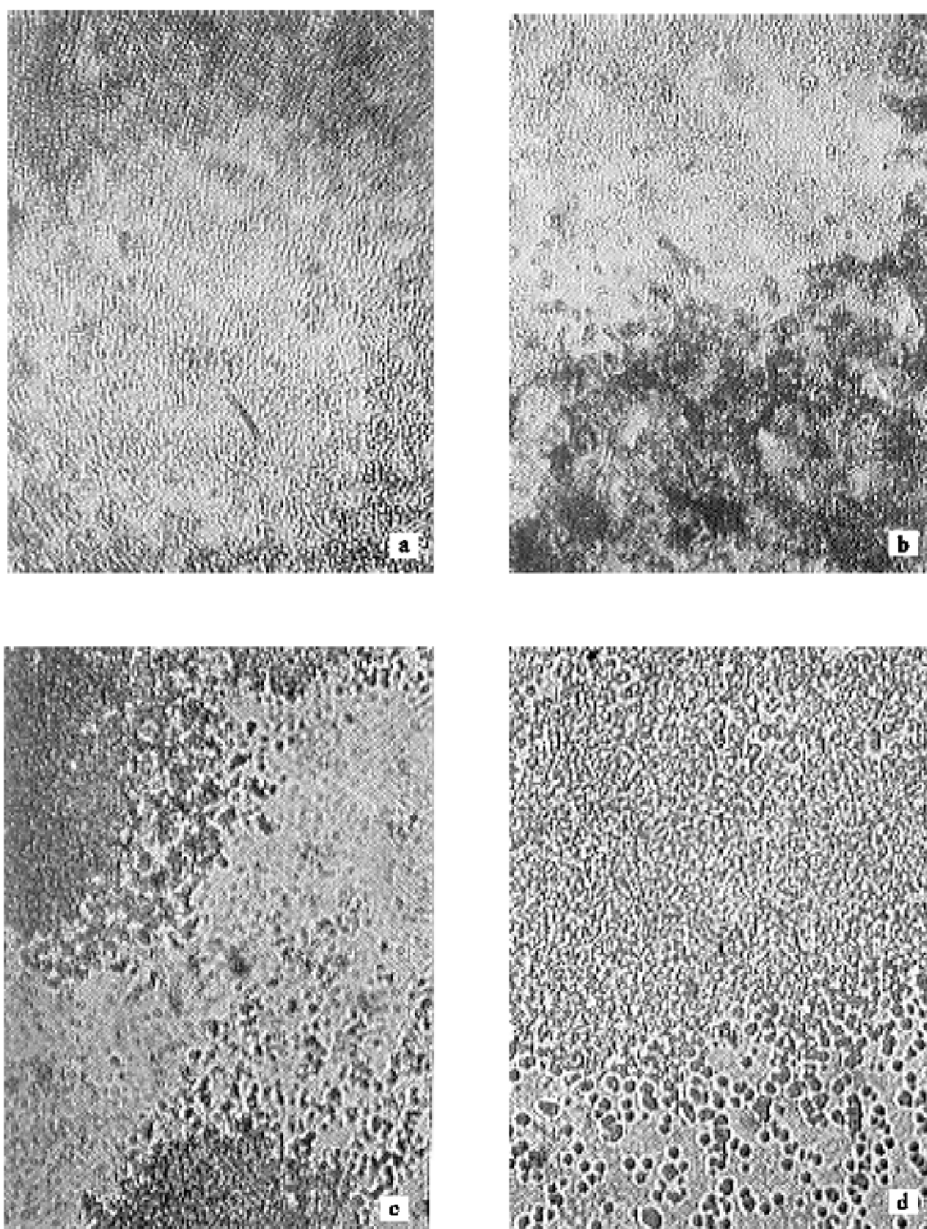


Fig. 6. Microscopical view of CC/CEF monolayers under agar: a. a whole plaque (32 $\times$) and b. a part of a plaque (160 $\times$) following inoculation with Bulgarian A/PMV-1 pigeon isolates; c. haemadsorption of RBC after the removal of agar layer of CC/CEF infected with local pigeon A/PMV-1 isolates (63 $\times$) and d. the same at 160 $\times$.

Cell monolayers with plaques adsorbed the added chicken RBC after the removal of the cover agar layer despite the pathotype of the viral strain. The microscopy revealed that adsorbed RBC covered completely plaque configurations or (in Petri dishes with extremely virus-destructed cellular monolayer) appeared diffusely disseminated on fused plaques.

The elution of adsorbed RBC at 4°C

was of a slow type, i.e. the sample remained unchanged for more than 24 h.

The agglutination activity of pigeon A/PMV-1 strains against chicken erythrocytes was well expressed – haemagglutination titres ranged from 1:640 to 1:1280 (Fig. 7). When heated to 56 °C, haemagglutinins were thermostable and remained active from 60 to 120 min (Fig. 8).

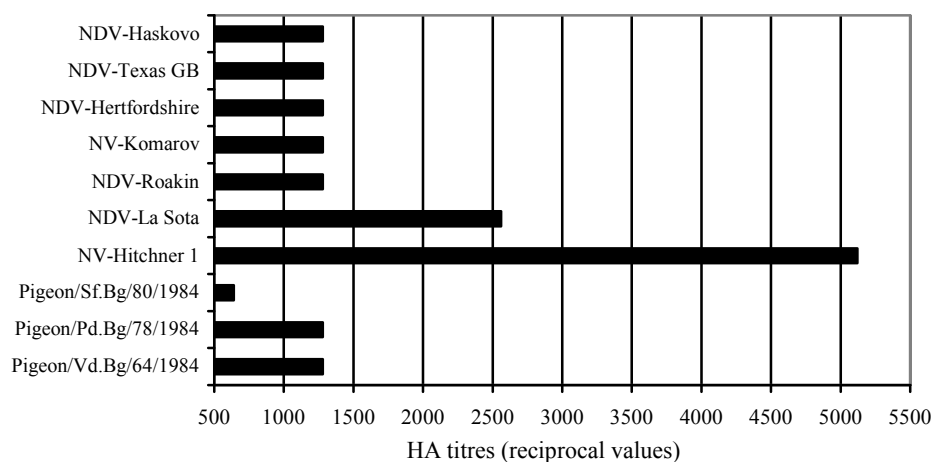


Fig. 7. Haemagglutination activity (HA) of three Bulgarian A/PMV-1 strains from pigeons and reference NDV strains.

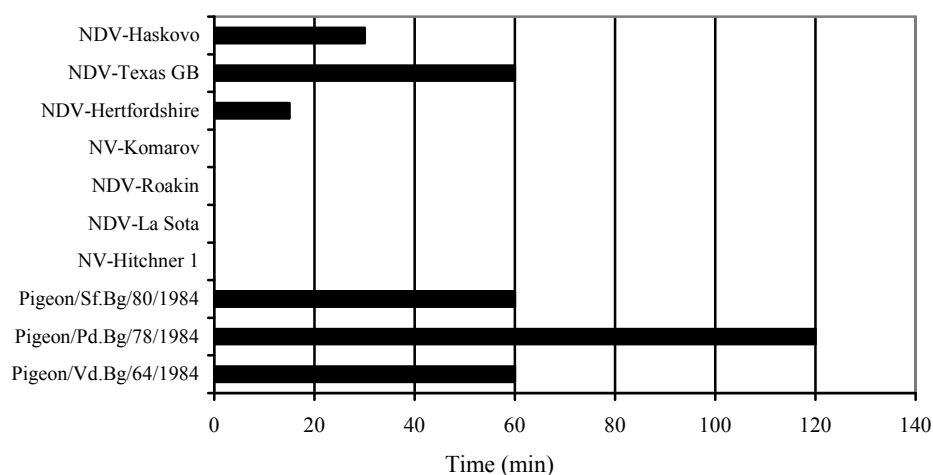


Fig. 8. Thermostability of the haemagglutinins of three Bulgarian A/PMV-1 strains from pigeons and reference NDV strains.

DISCUSSION

It is recognized that the virus, causing pigeon paramyxovirus type 1, is a mutant of NDV, adapted solely to this avian species (Vieane et al., 1983). It is also termed as a variant NDV strain (Alexander and Parsons, 1984; 1986a, 1986b; Engström et al., 1985; Alexander et al., 1986a; Lister et al., 1986) that is less dangerous for the *Gallinaceae* family (Alexander et al., 1984a; Anonymous, 1984b; Hadjiev, 1988; Sanford and Hampson, 1989; Buonavoglia et al., 1991; King, 1996). Alexander and Parsons (1984, 1986a) hypothesized that there were differences in the specificity of pigeon proteases from chicken ones and that this resulted in selection of NDV populations in pigeons, sensitive to pigeon proteases. The differences in the pathogenicity of pigeon A/PMV-1 strains, tested in chickens were probably due, in their opinion, to the selection of separate branches of viruses from the heterogeneous population that were more or less able to reproduce in chicken tissues as well. The fact that a substantial number of wild avian species are NDV-sensitive (Lancaster and Alexander, 1975) creates a prerequisite for persistence of this virus in avian species that are less susceptible in natural conditions but favourize mutagenic processes and as a consequence, the creation of new viral variants, such as pigeon A/PMV-1 (Alexander et al., 1985b). Therefore, the risk for readaptation of the virus for birds from the *Gallinaceae* family following a direct (Sabban et al., 1982) or indirect (Anonymous, 1984a) contact with pigeons, carrying the A/PMV-1, is not impossible and the hazard for a reversion in virus pathogenicity is real. This hypothesis, in our opinion, is satisfactory and could explain the substantial variations in the virulence of pigeon A/PMV-1 strains

isolated from various European regions, tested according to routine biological tests in chickens as well as the epizootics of a particular form of Newcastle diseases in chickens in Great Britain and Austria. Viral isolates from those foci showed all features of pigeon A/PMV-1 variants and of course, some differences from authentic NDV strains (Alexander et al., 1984a, 1985a; Anonymous, 1984a, Vasichek, 1985; Alexander and Parsons, 1986b).

Černik et al. (1985b) suggested that pigeon A/PMV-1 isolates in Czechoslovakia originated from the velogenic-viscerotropic NDV pathotype, whose pathogenicity for chickens was decreased to a degree, characteristic for mesogenic strains because of the multiple passages in pigeons. Saghy et al. (1985) refer pigeon A/PMV-1 isolates in Hungary to the mesogenic NDV pathotype as well.

The three Bulgarian pigeon A/PMV-1 strains refer as mesogenic reference NDV strains according to the results of most laboratory tests for typization (MDT/MLD, ICPI, IVPI, MDT/ICP) and as velogenic NDV strains according to the plaque-forming capacity in CC/CEF under agar and haemagglutinin thermostability. Our MDT values for local pigeon A/PMV-1 strains were similar to data for strains, isolated in other countries (Černik et al., 1985b; Sabban et al., 1982; Jestin et al., 1989; King, 1996); ICPI scores were comparable to those of similar strains from Great Britain (Alexander et al., 1985a), Belgium (Meulemans et al., 1984) and other countries (Alexander et al., 1985b; Shakal, 1989; Singh, 1991, 1993; Wernery et al., 1992) but different from those of other pigeons A/PMV-1 strains (Richter et al., 1983; Alexander et al., 1984b; 1985a; 1985b, Černik et al., 1985b, Kaleta et al., 1985; Lister et al., 1986; Kaleta, 1992; Capua et al., 1994;

King, 1996). To a certain extent, this is valid for IVPI scores as well – complete or partial match to some of reported data (Alexander et al., 1984b, 1985a, 1985b, Černík et al., 1985a) or disagreement with others (Alexander et al., 1985a; Engström et al., 1985; Kaleta et al., 1985; Lister et al., 1986; Jestin et al., 1989; Capua et al., 1994; King, 1996).

EID₅₀ values of Bulgarian pigeon A/PMV-1 strains were higher than the corresponding ELD₅₀ values that is a characteristic feature of NDV strains of the lentogenic pathotype. On the contrary, the same values for velo- and mesogenic NDV strains were identical because each CE, infected by those viruses died always within the period of observation (Hadjiev, 1988).

Černík et al. (1985a) did not observe any clinical signs in 1-, 2-, 3- and 8-week-old chickens, experimentally infected with 9 pigeon A/PMV-1 strains isolated in 1983 from carrier pigeons in South Czechia, Moravia and West Slovakia, but in blood sera, in a like manner as our experiments, specific antihaemagglutinins in titres up to 1:256 were present. Haemagglutinin thermostability at 56°C was similar to that of the velogenic-viscerotropic reference NDV strain California 1082/71. Haemagglutinins of Bulgarian pigeon A/PMV-1 strains were also thermostable and remained active for more than 15 min at 56°C. Comparable results are reported also by King (1996).

Haemagglutination activity of Bulgarian pigeon A/PMV-1 strains (within the frames of 1-2 double dilutions) was completely identical or close to that of similar strains in Italy (Biancifiori and Fioroni, 1983) and Czechoslovakia (Černík et al., 1985b), but was higher than that reported by Engström et al. (1985), Stone (1989), Singh (1993) and King (1996).

Plaque-forming capability of Bulgarian pigeon A/PMV-1 strains in primary monolayer CC/CEF are, in our opinion, very useful, because, like other laboratory tests, they reveal some particularities (differences) compared to reference NDV strains.

On the basis of our results, we confirm the assumption of Singh (1993) and King (1996) that variant A/PMV-1 strains that caused the panzootic among domestic pigeons after 1982, were not limited within the frames of a single pathotype, oppositely to the authentic NDV strains.

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Paper received 06.12.2000; accepted for publication 11.04.2001

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