



*Review*

## REVIEW OF INFECTION WITH AVIAN PARAMYXOVIRUS SEROTYPE 2 (APMV-2) AND FIRST RESULTS OF BULGARIA

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### ABSTRACT

The place, antigenic diversity and nomenclature of APMV-2 are successively described. The methods of virus isolation, significance for avian pathology and global distribution of infection through serological, virological surveys and experiments are reviewed. The first investigations with avian blood sera in Bulgaria (n=253) originating from 8 farms from different parts of the country to detect antibodies against APMV-2 are outlined. The data showed spread of infection among hens and chickens with 14.53 % positive samples, and presence of infection in all surveyed farms.

**Key words:** Avian paramyxovirus – 2 (APMV-2), birds, infection, Bulgaria.

### INTRODUCTION

#### 1. Avian paramyxoviruses from genus *Avulavirus*

The Paramyxoviridae family from order Mononegavirales includes important avian representatives. They are classified in subfamily Paramyxovirinae, genus *Avulavirus*. *Avulavirus* consists of 9 (nine) serologically distinct paramyxoviruses termed Avian paramyxovirus 1-9 (APMV 1- 9) (1, 2, 3, 4). A tenth serotype - APMV10/penguin/Falkland Islands/324/2007 – is isolated but not definitively classified.

The nomenclature of APMV isolates is similar to that of influenza viruses (5). The name of the strain should contain: 1) the serotype; 2) the species or type of the original host; 3) the state or geographical region; 4) strain number (if any); 5) year of isolation. The “Yucaipa” virus refers to APMV-2, as the first isolate was named as APMV-2/chicken/California/Yucaipa/56 and is the prototype of this serological group.

Alexander (6, 7) presents one prototype strain of each APMV serotype outlining the most commonly affected hosts as well as other susceptible birds (**Table 1**).

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The most important avian pathogen is the Newcastle diseases virus (APMV-1). There is no evidence that APMV-2 could infect other species although it is isolated from monkeys (8).

#### 2. Avian paramyxoviruses serotype 2 (APMV-2).

In 1956 Bankowski et al. (9) isolated a paramyxovirus from chickens with respiratory disease affecting the larynx and the trachea. The isolate originated from Yucaipa, California, USA and was named APMV-2/USA(Ca)/Yucaipa/1956. The virus turned out to be serologically distinct from APMV-1 (NDV).

#### A. Serological investigations in birds

Serological tests carried out in different domestic and wild bird species have witnessed a wide spread of APMV-2 (10). Antibodies have been established in domestic fowl (chickens, turkeys, ducks, geese, ostriches, peacocks), in cage birds (parrots, pigeons, finches, sparrows) and other decorative and wild avian species (11). APMV-2 is detected in birds in Europe (so far, there are no reports for presence of APMV-2 in birds in Bulgaria), Asia, Africa, North and South America (12, 13).

Serological studies of domestic fowl in the USA showed that the virus infected more often turkeys than chickens (14, 15). In Spain, 14.7 % of laying hens (341 birds) and 39% of chickens (123 birds)

had antibodies against the virus. Antibodies have been reported in 43.7 % of farms with layers and 80% of chicken farms (1).

Serological tests of blood sera from chickens, ducks, peacocks, ostriches and parrots in China exhibited antibodies in 80% of parrots, 42–47% of ostriches, peacocks and chickens, and 25% of ducks (11).

**Table 1.** Prototype viruses and APMV hosts

Prototype viruses	Natural hosts	Other hosts
APMV-1 = Newcastle disease virus (NDV)	multiple avian hosts	
APMV-2/chicken/California/Yukaipa/56	turkeys, passerine birds	chickens, psittacine birds, rails
APMV-3/turkey/Wisconsin/68*	turkeys	-
APMV-3/parakeet/Netherlands/449/75*	psittacine birds, passerine birds	-
APMV-4/duck/Honk Kong/D3/75	ducks	geese
APMV-5/budgerigar/Japan/Kunitachi/75	budgerigars	-
APMV-6/duck/Honk Kong/199/77	ducks	geese, turkeys, rails
APMV-7/dove/Tennessee/4/75	pigeons	turkeys, ostriches
APMV-8/goose/Delaware/1053/75	ducks and geese	-
APMV-9/duck/New York/22/78	ducks	-

\* The types could be distinguished via serological tests with monoclonal antibodies

## B. Isolation and identification of APMV-2.

The sampling methods (cloacal, oropharyngeal, lung homogenates, intestinal homogenates and intestinal content) as well as isolation techniques (8-10-day-old chick embryos) are identical to those for APMV-1 (4).

Madhuri et al., 2010 (16) investigated the mean death times (MDT) of 9-day-old chick embryos and intracerebral pathogenicity index (ICPI) of day-old chicks and reported that MDT was over 168 hours, and ICPI – 0. In another study, Kim et al., 2012 reported MDT values over 144 h and ICPI – 0.

The haemagglutination activity of all nine APMV serotypes and all 16 avian influenza virus (AIV) subtypes is best shown with chick red blood cells. Serological test (immunodiffusion test, haemagglutination inhibition test) are used for detection of antibodies in blood sera and differentiation of APMV from AIV. Haemagglutination inhibition (HI) is a specific test for distinction of the nine APMVs using type-specific polyclonal sera. Neuraminidase inhibition tests (17, 18, 19, 20), serum neutralization test (20) or agar gel diffusion test (18, 21, 22, 23) did not result in serotype differentiation.

The HI test results in some cross reactions between serotypes (10), but Lipkind & Shihmanter, 1986 (24); Lipkind et al., 1986 (25) believe that they are sufficient for distinction. Low-titre cross reactions have been observed among APMV- 1 , -3 , -4 , -7 , -8 , and -9 as well as between APMV-2 and -6.

The isolation of APMV-2 from domestic fowl is less frequent than that of APMV-1 due to lack of purposeful investigations, although the virus has caused problems in chickens and turkeys on a global scale – the USA, Canada, Russia, Japan, Italy, Germany, Israel, India, Saudi Arabia, France, China, Costa Rica, Kenya, Senegal (Alexander, 1980; Alexander, 1985, Guo-zhong Zhang et al, 2006).

The mandatory tests of quarantined imported cage birds often result in APMV-2 isolation, mainly from passerine and psittacine species (12, 26, 27). Strains have been also isolated from different species parrots, chaffinches, amadinas, finches, Eurasian wrens etc.

The attempts for isolation in freely living birds are also successful, most commonly from passerines and parrots, less frequently from mallards, coots, herons, birds of prey (10). In Senegal parrots for

export, APMV-2 was isolated from 3.7 % of tested birds (28). In Germany APMV-2 was detected in 31% of freely living passerine birds (29). For one year in the UK, APMV-2 was isolated from 38 out of 61 (62%) quarantined parrots (30). Over a 8-year period in the USA, APMV-2 was isolated from 46% of all quarantined birds. In southeastern Asia, 56% of all cloacal isolates were identified as APMV-2. The infection is the most prevalent between July and September, where young birds are available (31).

### C. Antigenic diversity

There is little information about the antigenic diversity of most APMV serogroups. The antigenic and structure diversity of APMV-2 is acknowledged (26, 32), and it does not reflect on their epidemiological and biological properties. Ozdemir et al., 1990 (33) studies 53 isolates with five types of monoclonal antibodies against haemagglutinin. Isolates were distributed in four groups according to their antigenic relatedness (**Table 2**). Later, Mahmood et al., 2010 (34) allotted them to 5 groups (80% homology) by means of sequence analysis of the HN gene of 22 isolates, with two subgroups to groups 2 and 4.

**Table 2.** Groups of APMV-2 isolates after monoclonal antibody test

Group	Avian isolates
First group	Isolates from parrots, some passerines, mallards, coots, turkeys
Second group	Isolates from chickens
Third group	Two passerine strains
Fourth group	More passerine strains

### D. Clinical, virological and serological investigations of APMV-2.

Health status data in psittacines and passerines with APMV-2 isolates vary within a broad range. Strains have been isolated from asymptomatic birds, birds with mild respiratory signs as well as subjects with severe pneumonia, mucoid tracheitis, diarrhoea, reduced activity and high mortality.

The incubation period in intratracheally infected birds is 4-6 days (14, 15).

Chickens and turkeys infected with isolates from cage birds (psittacines and passerines) did not exhibit clinical signs, but immune response (antihaemagglutinins) as well as microscopic changes in the respiratory tract and the pancreas are present (35). Mild respiratory signs were observed in an experiment with 7-day-old chickens and more severe symptoms resulted after co-infection with *Mycoplasma* or infectious bronchitis virus. APMV-2 is reisolated from the bursa of Fabricius, the trachea, lungs, thymus and more rarely, from the spleen and the kidneys (36). Madhuri et al., 2010 (16) investigated the clinical signs, seroconversion and virus localisation in 4-week-old chickens and turkey poults infected with chicken/California/Yukaipa/56 isolate from

Bangkok. The authors did not describe any

clinical signs, but the virus was detected in the respiratory and alimentary tracts with virus shedding. Seroconversion was established on the 6<sup>th</sup> day post infection.

Chickens infected with APMV-2, 4, 6 from wild birds (35) showed mild respiratory signs and microscopic lesions of trachea, lungs, stomach and pancreas. Antihaemagglutinins were induced only by APMV-2.

The virus is shed from the alimentary and respiratory tracts (37). In natural infections, the virus spread within the flock is slow and not all birds exhibit immune response (38). Spread between closely located flocks is occasionally present (39).

In chickens and turkeys, naturally infected with APMV-2, the virus had caused mild respiratory signs or birds were asymptomatic (14, 40, 41). More severe symptoms were reported in infected turkeys compared to chickens. Lang et al., 1975 (42) established severe respiratory signs, sinusitis, varying death rates and reduced egg production in APMV-2 infected turkeys. It is demonstrated that observed signs were more severe in cases where

other viral and/or bacterial agents of disease were also involved. The authors recommended depopulation of poultry flocks infected with the virus as a means of eradication of infection.

It was found out that the APMV-2 was widely prevalent among turkeys in Israel and provoked a respiratory disease at the background of complicated infection (43). In a field experiment, Bankowski et al., 1981 (15) demonstrated that APMV-2 reduced egg production in turkeys without affecting hatchability. There is evidence that APMV-2 could be spread vertically (44).

### 3. First reports for avian paramyxovirus-2 (APMV-2) infection in Bulgaria

Blood sera collected from poultry (136 turkeys; 291 hens and chickens) from 8 farms and 2 private owners from 10 settlements in 4 regions (Razgrad, Burgas, Stara Zagora, Kardzhali) were assayed for detection of post infection antibodies against APMV-2 (an APMV-2 strain from the National Diagnostic and Research Veterinary Medical Institute, Exotic and Emerging Diseases Lab, was used as antigen whose identity was confirmed at Instituto zooprofilattico sperimentale delle venezie – laboratorio virologia - Italia).

The haemagglutination inhibition test was carried out using the method approved by OIE, 2012 (4) with 8 haemagglutination units viral antigen. The results are shown in **Table 3**.

**Table 3.** Results from studied avian blood sera screened for antibodies against APMV-2

Avian species	Studied regions/ positive regions	Settlements	Studied farms/ positive farms	Studied samples/ positive samples
Turkeys	3/0	3	4/0	136/0
Hens and chickens	3/3	6	6/6	117/17

The results show that positive results were detected only in hens and chickens – 14.53% of all studied birds. All farms gave positive results for the infection. The results have shown the presence of APMV-2 infection among poultry in Bulgaria.

### CONCLUSIONS

Results show that in Bulgaria hens and chickens have infection with avian paramyxovirus serotype 2.

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