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Original Contribution

PREVALENCE OF AVIAN PARAMYXOVIRUS-2 AMONG DOMESTIC FOWL SPECIES IN BULGARIA IN 2013 AND 2014

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ABSTRACT

The haemagglutination inhibition (HI) test is used for testing 1258 blood sera from four avian species (hens and broilers, turkeys, partridges and geese) for detection of post infection antibodies against avian paramyxovirus -2 (APMV-2). The samples were collected from 14 regions in Bulgaria from fowl reared in 27 farms (commercial laying hens, commercial broilers, commercial laying turkeys and game breeding station with partridges) - 15, 9, 2, 1 samples, respectively. Another 11 samples were obtained from backyard poultry (laying hens, laying turkeys, laying geeses) – 8, 2, 1 birds respectively.

Positive results from tests were established in sera from backyard geese, from commercial broilers, commercial laying hens, and partridges (75%, 12.96 %, 7.81 % and 5.17 % respectively).

In studied farms with hens and broiler chickens, positive samples from broilers predominated (88.89 %), while the prevalence on laying hen farms was 66.67 %. Positive samples were detected in 10 (71.43 %) out of the 14 surveyed regions.

Antibody titres varied from 1:4 ($\log_2 2$) in hens, broilers and geese, 1:16 ($\log_2 4$) in partridges and attained 1:32 ($\log_2 5$) in broilers, 1:64 ($\log_2 6$) in geese and partridges and 1:128 ($\log_2 7$) in laying hens. Among geese and partridges, samples with titres 1:16 ($\log_2 4$) were predominating – 60 % and 66.67 %, followed by broilers with titres 1:8 ($\log_2 3$) – 37 % and hens with titres 1:4 ($\log_2 2$) – 47 %.

Key words: Avian paramyxovirus – 2 (APMV-2), Birds, Bulgaria, Infection

INTRODUCTION

The paramyxoviruses isolated from avian species have been classified by serological testing and phylogenetic analysis into ten subtypes designated APMV-1 to APMV-10 (1). For the first time, the virus was isolated in 1956 by Bankowski et al. (2) from chickens with acute laryngotracheitis and termed PMV–2/Chicken/California/Yuacapa/56. Later, the virus was isolated and serologically proved in other avian species, including wild birds mainly from *Pssittacine* and *Passeriformes* orders (3-6) as well as domestic fowl (chickens, turkeys etc.) reared in industrial farms or private backyards (7). 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 (4-7)].

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Serological tests carried out in different domestic species have witnessed a wide spread of APMV-2. Antibodies have been established in domestic fowl - chickens, turkeys, ducks, geese, ostriches, peacocks (5, 8-11).

In a survey of 168 chickens and turkey farms in the USA, (2, 12) established a higher prevalence of APMV-2 among turkeys (27 positive birds out of 249 studied – 10.84%) than among chickens (4/253 – 1.58%). In another study Warke et al. 2008 (10) found out 3 affected farms out of 29 commercial layer hen farms (10.34%), and 10 out of 47 chicken farms (21.28%).

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 (8). Serological tests of blood sera from chicken flocks in China demonstrated that all flocks were positive for APMV-2. The prevalence of positive birds in different flock types – layer hen breeders, stock layers, meat-type breeders, broilers were 48.6%, 23.5%, 59.5%, 11.7%. The distribution of results according to the breed (from a total

of 9 studied breeds) varied from 90.4% for Hubbard broiler chicken to 23.1% in Lohmann layer chicken. A total of 16 regions were studied, with positive results in 14 of them. The averaged seropositivity of APMV-2 for chickens, ducks, peacocks, ostriches, and partridges was 42,9%; 25,1%; 45,8%; 47,6%; and 80% respectively (9). In Saudi Arabia, 52.35% of birds at farms and 71.4% of backyard poultry were found to be positive for the virus. The age distribution of results exhibited negative results in birds <5 days of age, and positive – after 19 days of age. APMV-2 positive birds between 19–35 days of age were 58.33%, and those between 25 and 62 weeks of age: 47.05% (11).

On the basis of blood serum tests, we aimed to report the first occurrence of APMV-2 and its prevalence in domestic fowl species reared at farms or private yards in Bulgaria.

MATERIAL AND METHODS

Virus. APMV-2 (Chicken/Yucaipa/Cal/56 strain) was obtained from the National Diagnostic and Research Veterinary Medical Institute, Exotic and Emerging Diseases Lab, and was used as antigen whose identity was confirmed at Instituto zooprofilattico sperimentale delle venezie – laboratorio virologia – Italia. The virus was passaged after inoculation via the allantoic route in 9-day chicken embryos. The allantoic fluid with APMV-2 was collected 4 days post infection and tested in the haemagglutination (HA) test (13). After making two fold dilutions in saline, a 0.5% suspension of chicken erythrocyte was added. Allantoic fluid was stored at –80 ^{0}C until use.

Preparation of hyperimmune serum (positive and negative controls). Sera with anti-haemagglutinins against APMV-2 were obtained after inoculation of two 30-week-old chickens with APMV-2 antigen as described by Alkhalaf, 2009 (11). The serum was used for determination of the used virus as APMV-2 and as a confirmation test in case of positive results from haemagglutination inhibition test (HI) tests of patient sera.

Negative control serum was obtained from clinically healthy birds. The serum was used as negative test when positive results of patient sera were obtained from the HI reaction.

Serum collection. Blood samples were collected from birds belonging to 15 commercial hen farms, 9 broiler farms, 2 turkeys farms and one quail farm (a total of 27 farms). Samples from backyard birds were collected in 11 settlements (from hens in 8 settlements, from turkeys – in 2 and geese – in 1 settlement). The samples were collected in 2013 and 2014. The total number of samples was 1258, comprising 1031 birds from farms (590 from hens, 113 from turkeys, 270 from broilers, 58 from guinea fowl) and 227 owned by private owners (153 from hens, 54 from turkeys, 20 from geese). The tested birds were from 14 regions of the Republic of Bulgaria (Burgas, Blagoevgrad, Gabrovo, Dobrich, Kardzhali, Montana, Pernik, Pleven, Razgrad, Sofia district, Silistra, Stara Zagora, Sliven, Shoumen). Serum was extracted and stored at -20 ºC till further analysis.

Method of investigation and reporting of results.

The haemagglutination inhibition test (HI) was carried out using the method approved by Thayer and Beard, 1998 (14) with 8 haemagglutination units viral antigen. The tested serum samples were heated at 56°C for 30 minutes in a water bath to inactivate complements. β procedure (Diluted Serum-Constant virus) was performed in 96 well round bottomed microtiter plates. After making serial dilutions of the tested serum, antigen was added, incubated for 30 minutes and 0.5% chicken erythrocyte suspension was added. The plates were left at room temperature until the known HI- positive wells exhibited a tight, well-circumscribed button of unagglutinated, sedimented erythrocytes. The HI titer was recorded as the reciprocal of the highest dilution of serum at which there was complete inhibition of haemagglutination. The validity of the results was checked with negative control serum, which not give a titre 1:4 ($\log_2 2$), and a positive control serum for which the titre were 1: 256 ($\log_2 8$).

Analysis. Seropositive rates (SR) were calculated using the following formula: SR = (no. of sera positive/no. of serum samples) x 100 %. The geometric mean titer (GMT) and arithmetic means of the HI titres were used.

RESULTS

For the first time in Bulgaria is carried out to investigate the prevalence of APMV-2 in birds. The results from the tests showed that out of the 1258 blood sera, 111 were positive for post infection antibodies against APMV-2 (8.82 % of studied samples). The positive samples were from geese, hens, broiler chickens and partridges **(Table 1).** Positive samples from geese prevailed, followed by hens and broilers, and partridges. All samples from turkeys were negative.

Table 1. *Results for the occurrence of post infection antibodies against APMV-2 in tested birds*

№	Bird species	Number of tested sera	Number of positive sera	Seropositive rates
1	Geese	20	15	75.00
2	Hens and broilers	1013	93	9.18
3	Partridges	58	3	5.17
4	Turkeys	167	0	0.00

From birds reared at farms (hens, broiler chickens, partridges and turkeys), positive results were obtained in two species – hens and broilers, and partridges. The major part of positive sera was encountered in hens and broilers **(Table 2).** From three backyard birds species (hens, turkeys, geese), only geese turned out to be APMV-2 positive with high prevalence rate of 75%. The age distribution of tested birds showed predominance of positive results among young birds with difference of 5.15% (12.96 % and 7.81 %).

Table 2. *Results for the occurrence of post infection antibodies against APMV-2 in tested birds with respect to the rearing type*

№	Rearing type	Avian species	Number of tested sera	Number of positive sera	Seropositive rates
1	Farm	1. Broilers	270	35	12.96
		2. Hens	590	58	9.83
		3. Partridges	58	3	5.17
		4. Turkeys	113	0	0.00
2	Backyard	1. Geese	20	15	75.00
		2. Hens	153	0	0.00
		3. Turkeys	54	0	0.00

The positive farms with young birds (broiler chickens) were more than farms with adult birds (hens) – 88.89% vs 66.67% **(Table 3).**

Table 3. *Results for the occurrence of post infection antibodies against APMV-2 in tested hens and chickens with respect to the rearing type*

№	Rearing type	Tested farms/settlements with backyard fowl	Positive	Percentage
1	Farms with hens	15	10	66.67
	Farms with broilers	9	8	88.89
2	Backyard hens	8	0	0.00

Out of the studied birds from 14 regions, positive results were encountered in 10 regions (71.43%) – **Table 4.** The positive results varied from 100% to 80% according to the species. Sera from 4 regions were negative for APMV-2.

Table 4. *Results for the occurrence of post infection antibodies against APMV-2 in tested birds from the different regions*

Bird species	Number of studied regions*	Number of positive regions	%
Geese	1	1	100
Partridges	1	1	100
Hens	11	8	80
Broilers	5	4	80
Turkeys	4	0	0

Legend: * there are regions where several species or categories of birds have been tested

Anti-haemagglutinin titres in hens, broilers, partridges and geese varied from 1:4 ($\log_2 2$) to 1:128 ($\log_2 7$). In hens, titres 1:4 ($\log_2 2$) were most commonly seen – 47%. The prevalence of titres from 1:8 ($\log_2 3$) to 1:128 ($\log_2 7$) showed a progressive reduction, and only 3% of tested sera had titres of 1:128 ($\log_2 7$). Among broilers, birds with titres 1:8 ($\log_2 3$) – 37% were predominating, followed by 1:16 ($\log_2 4$) – 31 %, 1:32 ($\log_2 5$) – 20% and 1:4

($log_2$2) – 11% positive partridges had titres of 1:16 ($log_2$4) – 66.7 % and 1:64 ($log_2$6) – 33.3%. Positive sera from geese were with tires within the range from 1:4 ($log_2$2) to 1:32 ($log_2$5) with titres 1:16 ($log_2$4) being most numerous – 60%, while titres 1:32 ($log_2$5) and 1:64 ($log_2$6) were by 7% lower and titres 1:4 ($log_2$2) and 1:8 ($log_2$3) – by 13% lower.

DISCUSSION

Investigations with antibody detection have shown that APMV-2 spread could be wider than confirmed via isolation virus, so we choose this method of examination. The results demonstrated that APMV-2 infection was present among poultry in Bulgaria similarly to many other countries in the world. Out of the studied four bird species (hens and broiler chickens, turkeys, partridges and geese) post infection antibodies were detected in three: hens and broiler chickens, partridges and geese. Interesting results were obtained from backyard bird samples – geese were seropositive and hens and turkeys – seronegative. These results may provide useful data for APMV-2 epidemiology and have implications for poultry industry in Bulgaria.

Data for isolation and serological identification of APMV-2 infection (3, 5-6, 8-11, 15-16) are available in many wild and domestic bird species, but only we confirmed the presence of seropositive partriges proof in China.

Our results from the tests in chickens and turkeys differ from data obtained in the USA, Canada, Israel (12, 16-17) showing predominance of the infection in turkeys and at a lesser extent, in chickens and the occurrence in turkeys only in Italy and France (16). We proved the presence of antibodies in broiler chickens and negative results in turkeys regardless of the rearing technology.

Our data about the occurrence of the infection in different avian species and categories of birds (hens and broilers), reared under different conditions (in farms and backyards), prevalence rate and the uneven distribution in the country were compared to data from the USA, Spain, China, Saudi Arabia (5, 8-12, 17). Our results were comparable to those reported in the literature with respect to some parameters yet different with regard to others. For instance, the examination of adult (hens) and young birds (broiler chickens) in Spain (9), Saudi Arabia (11) and in the present results showed preponderance among young birds unlike data from the USA (2,10) and China (9). Nevertheless, with respect to the percentage of seropositive young birds, our prevalence of 12.96% was closer to the rate of 11.7% observed in China (9) but considerably different from markedly higher prevalence in Saudi Arabia 58.33 % (12), Spain 39 % (8) and very low occurrence – 1.58% in the USA (2,10). As adult birds are concerned, the prevalence in layer hens reared at farms was low (9.83%), similar to the rate of 14.7% from Spain (8) but unlike rates in stock layers from China – 23.5 % (9) and Saudi Arabia – 47.05% (11).

Surveys of the seroprevalence on poultry farms (hens and/or chickens) have been performed in the USA, Spain, China (8-10). Our results in hens (66.67%) were substantially higher by 10.34 % that the results in the USA (10) and by 43.7 % than the prevalence rate in Spain (8). In broiler chicken flocks, the positive results varied from 21.28% in the USA (11), 80% in Spain (8) and 100% in China (9); our rate of 88.89 % being comparable to Spanish data. In all cited reports, the prevalence among young birds was always higher.

Only one report from Saudi Arabia (11) presents data about the distribution of seroprevalence at farms and backyards, and the regional distribution of APMV-2 virus spread was commented in a survey in China (9) (16 regions studied; positive results in 14 or 87.5%). While in Bulgaria, the virus was not detected in backyard hens but only on farms, APMV-2 has been reported on both places in Saudi Arabia with higher prevalence in private-owned birds – 71.42% vs 52.35% on farms (11). The presence of seropositive hens and broilers on farms in our country in 2013 and 2014 was presumably due to maintenance and circulation of APMV-2 after introduction of new batches. In our country as well as in China, the virus was widely spread in the different regions. The negative results in some locations could be attributed to the insufficient number of collected samples.

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