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RESEARCH ARTICLE

Seroprevalence of Avian Reovirus Infections in Chickens in Western Provinces of Turkey

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Summary

In this study, seroprevalence of avian reovirus (ARV) infections in broiler and broiler breeder chicken farms in Aydin, İzmir and Manisa provinces were investigated. Serum samples (n = 631) collected from 8 broiler breeder and 18 broiler flocks were tested for ARV antibodies using a commercially available ARV Enzyme Linked Immunosorbent Assay (ELISA) kit. The sampled flocks were not vaccinated against ARV. The blood samples were taken at the time of slaughtering. Antibodies were found in all flocks tested; 224 of the 295 samples (75.93%) collected from broiler and 322 of the 336 samples (95.83%) collected from the broiler breeder chickens were seropositive. In total, 546 of the 631 serum samples (86.53%) were seropositive. Lameness and respiratory system disorders were observed in all of the broiler flocks but none of the broiler breeder flocks. Seropositivity in Izmir province (91.48%) was higher than seropositivity in Manisa and Aydin provinces (74.59 and 70.58%, respectively). These results suggest that ARV infections which may cause lameness, serious respiratory system disorders, and decreased productivity in broilers are very common in chickens in the Aegian region of Turkey. The infection is often subclinical in broiler breeders. These results highlight the need for regular implementation of programs to combat the ARV infection in western Turkey.

Keywords: *Avian reovirus, Broiler, ELISA, Seroprevalance*

Türkiye'nin Batı İllerindeki Tavukçuluk İşletmelerinde Avian Reovirus Enfeksiyonunun Seroprevalansı

Özet

Bu çalışmada, Aydın, İzmir ve Manisa illerindeki damızlık ve broiler yetiştiren tavukçuluk işletmelerinde Avian Reovirus (ARV) enfeksiyonunun seroprevalansı araştırıldı. Ticari Enzyme Linked Immunosorbent Assay (ELISA) kullanılarak, 18 broiler ve 8 broiler damızlık kümesine ait tavuklardan alınan toplam 631 serum örneği ARV antikorları yönünden test edildi. Kan örnekleri tavukların kesimi esnasında alındı. Örneklenen sürüler ARV enfeksiyonuna karşı aşısızdı. Bütün kümeslerde ARV antikorları bulunmuş olup, broiler kümeslerinden toplanan 295 örneğin 224'ü (%75.93) ve damızlık broiler kümeslerinden toplanan 336 örneğin 322'si (%95.83) seropozitif olarak saptandı. Test edilen toplam 631 serum örneğinden 546'sı (%86.53) pozitif olarak kaydedildi. Örnekleme esnasında, damızlık sürülerde solunum ve topallık belirtilerine rastlanmazken, broiler kümeslerinin tamamında bu problemler dikkati çekmekteydi. Seropozitiflik oranlarının İzmir ilinde (%91.48), Manisa ve Aydın illerine nazaran daha yüksek olduğu tespit edildi (sırasıyla %74.59 ve %70.58). Sonuç olarak, broiler yetiştiriciliğinde önemli solunum sistemi semptomlarına, topallığa ve verim düşüklüklerine yol açan reovirusların Ege Bölgesi'nde çok yaygın olduğu görüldü. Özellikle damızlık sürülerde enfeksiyonun yüksek prevalansa sahip olduğu ve çoğu zaman semptom göstermeksizin, gizli bir şekilde devam ettiği belirlendi. Bu araştırmanın sonuçlarına dayanarak, söz konusu virus enfeksiyonuna karşı düzenli mücadele programlarının hazırlanması ve uygulanması gerektiği sonucuna varıldı.

Anahtar sözcükler: *Avian reovirus, Broiler, ELISA, Seroprevalans*

INTRODUCTION

Avian reoviruses (ARV) are classified within the *Orthoreovirus* genus of *Reoviridae* family. There are 11 known serotypes [1] that differ substantially in their host specificity, and antigenic and biological properties [2,3].

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ARV infections cause serious economic losses in the poultry industry [4]. They are the cause of viral arthritis syndrome, also known as tenosynovytis, characterized by joint swelling and lesions of the gastrocnemius tendon. Inflammatory changes in tarsometatarsal joints and nearby tendon sheaths results in joint swelling [4,5]. Rupture of the gastrocnemius tendon and degeneration of the articular cartilage may occur in the course of serious forms of the disease and may cause bleeding within the joints resulting in greenish coloration of the skin. The poor appearance of the affected joints results in increased wastage in slaughterhouses [4,6]. If both of the joints are affected, the sick animals can not move and reach the feed, resulting in reduction in feed intake and growth retardation and, in some cases, death. Viral arthritis is rarely seen in animals younger than 4 or 5 weeks. The disease is mainly seen in broiler chickens but turkeys and lighter poultry breeds are also susceptible [4-7].

The avian reoviruses, either alone or in conjunction with other pathogenic bacteria, cause a range of additional disorders including enteric-respiratory diseases, myocarditis, hepatitis, malabsorbtion, helicopter disease, brittle bone disease, and femoral head necrosis [4,8]. Reovirus infections suppress the immune system in the poultry and maybe clinically asymptomatic [4-10]. The ARV infections are common all over the world [4,7]. The virus, as well as antibodies against it, has been isolated from poultry enterprises in various regions of Turkey either alone or along with other infectious agents but there are no recent studies to determinate prevalence with a reasonably large sample size [11-16].

The immune response raised against ARV is an important tool in diagnosis of the infection to determine proper time of vaccination which may prevent the disease and economic losses. Enzyme-Linked-Immunosorbent Assay (ELISA) has been reported as a sensitive and rapid test method for detection and quantification of antibodies against ARV by various investigators [17,18]. In this study, seroepidemiology of the ARV infections in broiler and broiler breeder enterprises in Izmir, Aydin, and Manisa provinces of Turkey, were investigated.

MATERIAL and METHODS

Samples **-** Blood samples were collected, at the time of slaughtering, from 631 chickens randomly selected from 18 broiler and 8 broiler breeder flocks from different farms located in Aydin, Izmir, and Manisa provinces of Turkey in year 2009. Broiler breeders were 13 and 66 weeks old whereas broiler chickens were 40-44 days old. The chickens in farms where samples were taken were not vaccinated against ARV infection. Serum was obtained by centrifugation of blood samples at 1700 x g for 10 min.

Enzyme Linked Immunosorbent Assay (ELISA) **-** Serum samples were screened for ARV antibodies using a commercially available ELISA kit (Avian Reovirus Antibody Test Kit, BioChek, Gouda, The Netherlands). The test was performed following the manufacturer's instructions in the Department of Microbiology, Faculty of Veterinary Medicine, Adnan Menderes University. In brief, the samples were diluted 1:500 in PBS and added on microtitre plates pre-coated with inactivated ARV-antigen. Antibodies present in the sample were captured by the antigen coated on the plate. Stringent washing was performed to wash away unbound antibodies and non-specific serum proteins. Anti-chicken IgG labelled with alkaline phosphatase was added on the wells to detect chicken anti-ARV antibodies originally bound to the antigen. After another wash to remove excess conjugate, pNPP chromogen was added to develop a yellow color, intensity of which is directly related to the ARV antibody concentration in the sample. The antibody titers were measured at 405 nm using an ELISA reader (Biotek Absorbance Microplate Reader, ELx808). The relative amounts of antibodies in chicken samples were calculated in comparison to the positive control. Final antibody titers were obtained using BioChek Software programme (Gouda, The Netherlands). Results of antibody titers were analyzed using T-test in SPSS programm [19].

RESULTS

In this study, antibodies against ARV were found in all flocks tested (100%). Two hundred and twenty four of the 295 samples collected from the broilers (75.93%) and 322 of the 336 samples (95.83%) collected from broiler-breeder chickens were seropositive. The average coefficient of variation for seropositivity in broiler breeders and broilers were 53 and 42%, respectively. Seropositivity rates in the Aydin, Manisa, and Izmir provinces were 70.58% (36/51), 74.59% (91/122), and 91.48% (419/458), respectively. In total, 546 of the 631 serum samples tested (86.53%) were seropositive *(Table 1)*. During sampling, lameness and respiratory system disorders were found in all of the broiler flocks but none of the broiler breeder flocks.

Antibody titers in the positive samples collected from broiler pens ranged from 1289 to 10227 (average 3882) whereas those collected from broiler breeders ranged from 1953 to 16997 (average 10699). Therefore the antibody titers were in broiler breeder flocks were significantly higher than those in broiler flocks ($P<0.001$) *(Fig. 1)*. Antibody titers in broiler flocks were more variable. Ten of the 18 broiler flocks tested had lower antibody titers than the remaining 8 flocks.

DISCUSSION

Avian reoviruses have been implicated in a range of poultry diseases including viral arthritis/tenosynovitis,

Table 1. *Seropositivity against avian reoviruses in broiler and broiler breeder chickens in Aydin, Izmir, and Manisa provinces*
Tablo 1. *Aydın, İzmir ve Manisa illerindeki broiler ve broiler damızlık tavuklarda avian reovirusa karşı seropozitiflik oranları*

Province	Broilers			Broiler Breeders			Total		
	Samples Tested	Seropositive Samples	% Seropositivity	Samples Tested	Seropositive Samples	% Seropositivity	Samples Tested	Seropositive Samples	% Seropositivity
Aydın	51	36	70.6	0	0	0	51	36	70.58
İzmir	162	125	77.2	296	294	99.3	458	419	91.48
Manisa	82	63	76.83	40	28	70	122	91	74.59
TOTAL	295	224	75.93	336	322	95.83	631	546	86.53

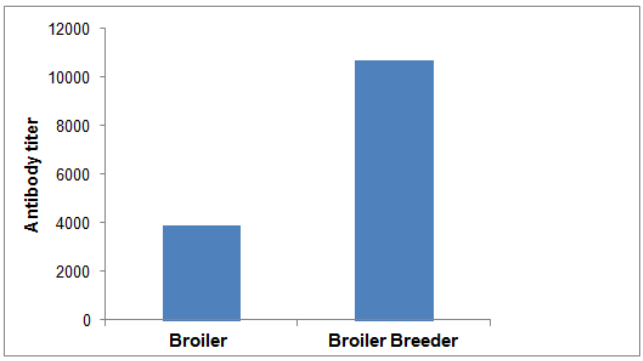

Fig 1. Mean antibody titers in samples taken from broiler and broiler breeder flocks

Şekil 1. Broiler ve broiler damızlık kümeslerinden alınan örneklerin ortalama antikor titreleri

respiratory disease, enteric disease and growth retardation/ malabsorption syndrome [4-10].

Avian reovirus infections are common all over the world, and may cause serious economic losses in the poultry industry. A seroprevalence study of egg-laying hens in China showed the presence of ARV infections in over 92% of unvaccinated chickens from different farms [20]. In another study conducted in Iran, the authors used ELISA and detected antibodies against ARV in 98.3% of the 572 samples collected [21]. Biswas et al.[22] reported that 56% (55/99) of the pens and 47% (138/295) of chickens were seropositive in a study conducted in Bangladesh.

Data on serological prevalence of ARV infections in various regions of Turkey, on the other hand, is very limited. Little research has been performed previously and there is no current and adequate scientific data in Turkey available. The extent of economic losses due to ARV infection is unknown. The virus had been identified, either alone or as a part of mixed infections [11-16] but there are no current data on the prevalence of reoviruses. Çöven and Çarlı [14] isolated the virus, mixed with adenovirus and Newcastle disease virus, in a flock suspected of being infected with Gumboro disease in various provinces in Turkey. ARV was isolated from 105 of the 174 bursa of Fabricius samples (60.3%) after passages in chicken embryo fibroblast cultures. Öztürk and Çöven [15] detected ARV in 3 of the 81 samples collected from 30 to 36 day old chicks from four broiler enterprises suspected of being infected with infectious bursal disease virus (IBDV). Akalın and Ergün [12] investigated the presence of antibodies against adenovirus and ARV in 600 samples collected from 11 flocks of broiler chickens with mal-absorption syndrome and 100 samples from a flock of broiler breeder chickens without clinical symptoms. The samples were tested using Agar Gel Precipitation Test (AGPT). No precipitating antibodies against neither of the viruses were found in any of the serum samples of 11 broiler flocks. However, precipitating antibodies against ARV and adenovirus were found in 11% and 9% of broiler breeders, respectively. Mutlu and Yiğit [16] reported that the antigenic characteristics of different ARV strains isolated in Turkey were very different and their antigenity did not resemble that of the S1133 strain that was frequently used for vaccination.

In this study, seroprevalence ARV infections in broiler breeder and broiler enterprises in Izmir, Aydin and Manisa provinces of western Turkey were investigated using ELISA. Serum samples (n= 631) collected from chickens in 18 broiler and 8 broiler breeder flocks were tested for ARV antibodies. Five hundred forty six (86.53%) of the 631 serum samples tested were seropositive. ARV antibodies were found in all flocks sampled. Rates of seropositivity were higher in broiler breeders than the broilers (75.93 vs 95.83%). Seroprevalence was higher in Izmir province than the other two provinces but this could be due to the larger number of samples collected from the Izmir province.

These data suggest that ARV infections are highly prevalent in chickens in the Aegean region of Turkey, especially in broiler breeder flocks which constitute a stock for poultry breeding. The seroprevalence of ARV found in this study was significantly higher than previously thought [11,12].

The most significant disease directly caused by ARV in chickens and turkeys is arthritis/tenosynovitis. Swelling and inflammation of the tarsometetarsal joint and the tendon sheath nearby may result in lameness and acute paralysis [4]. In addition, ARV infections may cause serious respiratory problems in poultry.

Although a detailed clinical investigation was not performed in this study, it was observed lameness and respiratory problems in all broiler flocks, but not in broiler breeder flocks. It is possible that older animals develop

age-related resistance to infection [23,24]. Young animals are more susceptible to ARV infections probably because their immune system is not yet fully developed [25].

In this study, antibody titers in samples taken from broiler breeders were higher than those taken from broilers. It is possible that the broiler breeders were being constantly re-infected with the virus subclinically, resulting in long-term production of antibodies. No signs of disease were noted in these older animals probably because they had higher antibody titers and fully developed immune systems. The fact that the ARV infection was found in all flocks tested shows the high virulence of the ARV infections.

In summary, ARV infections that may result in significant economic losses in poultry industry are very common in chickens in western Turkey. The virus probably circulates in broiler breeder flocks subclinically but causes disease in broilers. Current data on the presence and prevalence of ARV infections in Turkey is limited and there are no regular combat programs against the infection. The effectiveness of vaccination is unknown. Results from this study highlight the urgent need for seroepidemiologic, virological, and molecular research to develop and implement programs to combat the ARV infections.

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