



A Serological Investigation of Avian Reovirus Infections in Poultry Enterprises in Balıkesir and Bolu Provinces in Turkey*

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SUMMARY

Avian reovirus (ARV) is the cause of viral arthritis syndrome. In addition, ARV alone or in combination with other agents may cause clinical manifestations such as enteric respiratory disease, myocarditis, hepatitis and growth retardation/malabsorption syndrome in chickens. The objective of this study was to investigate the seroprevalence of ARV antibodies in broiler and broiler-breeder chickens in Balıkesir and Bolu provinces, the two important poultry breeding sites in Turkey. A total of 920 serum samples were collected from chickens vaccinated or unvaccinated against ARV in 11 broiler and 6 broiler breeder farms. ARV antibody titers were determined using a commercial Enzyme Linked Immunosorbent Assay kit (Avian Reovirus Antibody test Kit®, BioChek, Gouda, The Netherlands). ARV antibodies were detected in all poultry enterprises tested. When broilers were considered, 78% (156/200) of vaccinated and 84.18% (266/316) of the non-vaccinated chickens were seropositive. These ratios were 86.5% (173/200) and 89.71% (183/204) in broiler breeder chickens, respectively. Non-vaccinated chickens had higher seropositivity rates than vaccinated chickens when broiler chickens in Balıkesir were considered (92.03% vs 78%, $P<0.001$). However, seropositivity in non-vaccinated and vaccinated chickens were similar in broiler breeder chickens in Bolu (93% in vaccinated vs. 89.71% in non-vaccinated chickens, $P=0.35$). When chickens from the same province were compared, seropositivity in broiler and broiler breeder chickens in Balıkesir were similar (78% vs. 80%, $P=0.69$). However, among non-vaccinated animals in Bolu, seropositivity was higher in broiler breeder than the broiler chickens (78.09 vs 89.71, $P<0.01$). These results suggest that ARV infections, which cause significant economic and production losses in poultry, are very common in the Bolu and Balıkesir provinces. High seropositivity rates in non-vaccinated chickens suggests that vaccination practices are irregular in these provinces. Therefore, virological and molecular epidemiological studies should be carried out to control and combat the ARV infections.

Key Words: Avian reovirus, Broiler chickens, Broiler breeder chickens, Seroprevalence, Antibody, ELISA

ÖZET

Türkiye'nin Balıkesir ve Bolu İllerindeki Tavukçuluk İşletmelerinde Avian Reovirus Enfeksiyonunun Serolojik Olarak Araştırılması

Avian reovirüsleri (ARV) viral artrit sendromunun nedenidir. Ayrıca, ARV tek başına ya da diğer ajanlar ile birlikte, enterik solunum yolu hastalıkları, miyokardit, hepatit ve tavuklarda büyüme geriliği/malabsorpsiyon sendromu gibi klinik tablolara da neden olabilir. Bu çalışmada, Türkiye'nin önemli kanatlı yetiştiriciliği merkezlerinden olan Balıkesir ve Bolu illerindeki broiler ve broiler damızlık yetiştiren tavukçuluk işletmelerinde ARV enfeksiyonunun seroepizootiyolojik durumu araştırıldı. Ticari Enzyme Linked Immunosorbent Assay (ELISA) (Avian Reovirus Antibody test Kit®, BioChek, Gouda, Hollanda) kullanılarak 11 broiler ve 6 broiler damızlık yetiştiriciliği işletmesindeki ARV'una karşı aşı ve aşızsız tavuklardan alınan toplam 920 serum örneği ARV antikorları yönünden test edildi. Test sonucunda bütün kümeslerde ARV antikorları bulundu. Etçi tavuklar gözönüne alındığında, aşılanmış tavukların %78'i (156/200) ve aşılanmamış tavukların %84.18 (266/316)'inde antikor varlığı saptandı. Bu oranlar, aşılanmış ve aşılanmamış damızlık tavuklarda sırasıyla %86.5 (173/200) ve %89.71 (183/204) idi. Balıkesirdeki etçi tavuklar gözönüne alındığında, seropositivite aşılanmamış hayvanlarda aşılanmışlara oranla daha yüksekti (%92.03 ve %78, $P<0.001$). Ancak Bolu'daki damızlık tavuklarda aşılanmış ve aşılanmamış hayvanlar arası seropositivite oranları benzerdi (aşılanmış hayvanlarda 93% ve aşılanmamışlarda 89.71%, $P=0.35$). Aynı ilden toplanan örnekler karşılaştırıldığında, Balıkesir'den toplanan etçi ve damızlık tavuk örneklerindeki seropositivite oranı benzerdi (78% ve 80%, $P=0.69$). Ancak Bolu'daki aşılanmamış hayvanlar gözönüne alındığında, seropositivite damızlık tavuklarda etçi tavuklara oranla daha yüksekti (78.09 ve 89.71, $P<0.01$). Bu çalışmada elde edilen sonuçlar kanatlı endüstrisinde önemli ekonomik ve verim kaybına yol açan ARV enfeksiyonlarının Bolu ve Balıkesir illerinde çok yaygın olduğunu göstermektedir. Aşılanmamış hayvanlardaki yüksek antikor titresi bu illerde aşılama programlarının düzenli olarak uygulanmadığını düşündürmektedir. Bu yüzden ARV enfeksiyonlarını önlemek için virolojik ve epidemiyolojik çalışmaların başlatılması, mücadele programlarının geliştirilmesi ve hayvanların düzenli olarak aşılanmaları gerekmektedir.

Anahtar Kelimeler: Avian reovirus, Broiler, Broiler damızlık, Seropozitif, Antikor, ELISA

INTRODUCTION

Avian reovirus (ARV) infections cause serious economic losses in the poultry industry (Lu et al. 2015). They cause viral arthritis syndrome, also known as tenosynovitis, characterized by joint swelling and lesions in the gastrocnemius tendon (Jones 2000; Van Loon 2001). Affected joints may appear defective and result in increased wastage in slaughterhouses (Kibenge et al. 1982).

ARVs also may cause many additional symptoms such as enteric-respiratory diseases, myocarditis, hepatitis, malabsorption, brittle bone disease, and femoral head necrosis either alone or in collaboration with other pathogenic bacteria (Jones 2000). Reoviruses may suppress the immune system in the poultry, and thus, may present asymptomatic in the clinic (Schat and Van Santen 2008).

Investigation of pathogens and their epidemiological status are important factors to succeed in combatting infectious diseases. ARV infections are common in Turkey and around the world (Çarlı et al. 1992; Akalın and Ergün 1995; Mutlu and Yiğit 1997; Erol and Şengül 2012; Bokaie et al. 2008; Pu et al. 2008, Biswas et al. 2009)

Since the economic losses due to ARV infections have not been adequately researched, essential measures to combat the infections have not been taken sufficiently in Turkey. The objective of this study was to determine the seroprevalence of the ARV infections in the broiler and broiler breeder chickens in the Balıkesir and Bolu provinces, the most important areas of poultry production in Turkey. In addition, seropositivity rates and antibody titers in vaccinated and non-vaccinated animals were compared to investigate regularity of vaccination practices and immunization levels in animals vaccinated under natural conditions in these provinces. Finally, seropositivity rates between broiler and broiler breeder chickens were compared.

MATERIALS and METHODS

Samples

Blood samples were collected from 920 chickens randomly selected from 11 broiler and six broiler breeder farms located in the Balıkesir and Bolu provinces between September 2011 and May 2012. The experimental design used in this study is shown in Figure 1. The chickens were categorized into the "vaccinated" and "non-vaccinated" groups. The broiler breeder hens were vaccinated for the maternal immunisation of broilers. The broiler breeder chickens in the Bolu province were vaccinated using a modified live vaccine (REOGUARD®L, Merial, Merial Select, Inc., Gainesville, USA) on day 6 and rapelin on week 6. The breeder chickens in Balıkesir were vaccinated first on the 1st to 3rd weeks and again on 10th to 12th weeks using a live vaccine (Nobilis® REO 1133, Intervet, Milton Keynes, UK). A second inactive vaccine (Nobilis® REO Inac, Intervet, Milton Keynes, UK) was administered from 18th to 19th weeks.

ELISA

Serum samples were tested for ARV antibodies using a commercial ELISA kit (Avian Reovirus Antibody Test Kit, BioChek, Gouda, The Netherlands). The test was performed following manufacturer's instructions.

Statistical Analyses

Chi square test was performed for the comparative analysis of seropositivity rates in the groups of chickens (Steel and Torrie 1980). Independent samples T test was used for comparison of antibody titers of vaccinated and non-vaccinated chickens). Both tests were performed in the SPSS 10.0 software package.

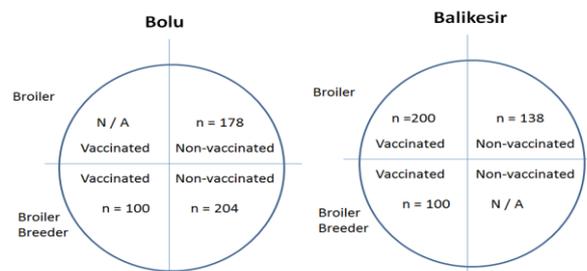


Figure 1. Experimental design used in this study

RESULTS and DISCUSSION

Avian Reovirus (ARV) infections are historically associated with a number of diseases such as viral arthritis/tenosynovitis, respiratory disease, enteric disease and developmental/malabsorption syndrome in the poultry industry (Menendez et al. 1975; Heide et al. 1981; Jones 2000).

Avian reoviruses are spread all over the world and can cause serious economic losses. Research studies investigating the existence and seroprevalence of ARV infections in Turkey are limited in the literature. In addition, there is not sufficient information on the economical losses caused by this infection in poultry breeding. The presence of the virus has been detected only in cases of mixed infections (Çöven and Çarlı 1997; Öztürk and Çöven 1997).

The objective of this study was to determine the presence and prevalence of ARV infections in broiler and broiler breeder chickens in the Balıkesir and Bolu provinces, two of the most important centers for chicken breeding in Turkey. Antibody titers and seropositivity rates in the vaccinated and non-vaccinated animals were compared to determine if vaccinations were performed regularly in the field and sufficient immunological response was obtained.

In this study, antibodies against ARV were detected in all enterprises (100%) sampled. Overall seropositivity was 84.56% (778/920) when data from all animals were considered. Overall results obtained in the study are summarized in Table 1.

In the Balıkesir province, seropositivity was 78% (156/200) in broiler chickens vaccinated maternally. This rate was 80% (80/100) in broiler breeder chickens. When vaccinated animals from both provinces were considered, seropositivity was 78% (156/200) in broiler and 86.5% (173/200) in the broiler breeder chickens. These ratios were 84.18% (266/316) and 89.71% (183/204) in broiler and broiler breeder chickens, respectively. When data from all animals were considered, 82.25% (329/400) of the vaccinated and 86.35% (449/520) of the non-vaccinated animals were seropositive.

Table 1. Seropositivity rates for avian reoviruses in the Balıkesir and Bolu provinces in Turkey

Production type	Vaccination		Province		Total	P
			Balıkesir	Bolu		
Broiler	Vaccinated	n	200	N/A	200	N/A
		Positive	156	N/A	156	
		Negative	44	N/A	44	
		%	78	N/A	78	
	Non-vaccinated	n	138	178	316	
		Positive	127	139	266	
		Negative	11	39	50	
		%	92.03	78.09	84.18	
BB	Vaccinated	n	100	100	200	<0.01
		Positive	80	93	173	
		Negative	20	7	27	
		%	80	93	86.5	
	Non-vaccinated	n	N/A	204	204	
		Positive	N/A	183	183	
		Negative	N/A	21	21	
		%	N/A	89.71	89.71	
^a Total	Vaccinated	n	300	100	400	<0.01
		Positive	236	93	329	
		Negative	64	7	71	
		%	78.67	93	82.25	
^a Total	Non-vaccinated	n	138	382	520	0.02
		Positive	127	322	449	
		Negative	11	60	71	
		%	92.03	84.29	86.35	

BB: Broiler breeders. ^aTotal includes both broiler and broiler breeder animals, N/A: No data were available, "%" represents percent seropositivity.

The seropositivity rates were comparable to those reported previously by Erol and Şengül (2012) in non-vaccinated chickens under natural conditions in the Ege province but higher than those reported by Çarlı et al. (1992) and Akalın and Ergün (1995). The health status and production traits in animals included in this study were not investigated. Therefore, it was not possible to determine effects of the infection on productivity traits and economic losses. However, results from the current as well as previous studies suggest that ARV infection could be causing significant economic losses in poultry, a significant food source in Turkey.

Comparison of the results from vaccinated vs. non-vaccinated animals is presented in Table 2. When broiler chickens in Balıkesir were considered, seropositivity was higher in non-vaccinated than the vaccinated animals (92.03% vs 78%, $P < 0.001$). However, seropositivity in vaccinated and non-vaccinated animals were similar among broiler breeder chickens in the Bolu province (93% vs. 89.71%, $P = 0.35$). These results suggest that seropositivity in the breeder broiler chickens was not influenced by vaccination. The reason for this observation could be that the natural infections are common in the region and the naturally produced antibodies might have neutralized the vaccine virus or that there are multiple serotypes of the virus in the region.

The results of this study also show that antibody levels in non-vaccinated animals could be higher than vaccinated animals. Possible reasons for this observation could be

that the virus that is prevalent in the region is virulent or that the ARV infection causes immunosuppression against the vaccine virus resulting in insufficient humoral response. The similar seropositivity in the vaccinated and non-vaccinated chickens suggest that vaccination programs in Turkey may not have been administered regularly.

Seropositivity in broiler and broiler breeder chickens are compared in Table 3. Seropositivity was not different in broiler and broiler breeder chickens when vaccinated animals in Balıkesir were considered (78% vs. 80%, $P = 0.69$). However, seropositivity was higher in broiler breeder than the broiler chickens when non-vaccinated animals in Bolu were considered (89.71% vs. 78.09%, $P < 0.01$). When data from all animals were considered, broiler breeder chickens had higher seropositivity than the broiler chickens (86.5% vs 78%, $P < 0.03$). However, the difference between broiler and broiler breeder animals was less (89.71% vs. 84.18%, $P = 0.07$).

The mean antibody titers have been presented in Table 4. It was first compared vaccinated vs. non-vaccinated animals. Non-vaccinated broilers had higher antibody titers than the vaccinated broilers in Balıkesir province (3369.3 ± 156.36 vs. 4613.4 ± 830.51), although the difference was statistically insignificant ($P > 0.05$). The high antibody titers in non-vaccinated animals suggest that the vaccination programs are not administered regularly in this region. Mutlu and Yiğit (1997) reported that there were many different antigens isolated from Turkey and

that the S1133 serotype used in vaccines was not related to some of those antigens. The effectiveness of vaccines against Reoviruses are not well known because of their large antigenic differences.

We then compared broiler vs. broiler breeder chickens from the same province. The antibody titers between broiler and broiler breeder chickens were similar in vaccinated animals in Balıkesir (3369.3±156.36 in broiler and 3895.2±255.29 in broiler breeder chickens). However, broiler breeder chickens had higher titers than the broiler chickens when non-vaccinated animals in Bolu were considered (5110.6±218.30 vs. 3287.0±180.47).

These results from Bolu province are in agreement with Erol and Şengül (2012) who reported higher infection rates in non-vaccinated broiler breeder than non-vaccinated broiler chickens. The authors also reported that the infection in broiler breeders are commonly subclinical. Our results suggest that subclinical infection in breeder chickens could be inducing long-term antibody production in these animals. In addition, it is possible that the broiler breeders are the main source of infection and, therefore, should be targeted in the initial stages of vaccination programs aimed at combatting the infection.

Mean antibody titers in the vaccinated and non-vaccinated broiler chickens were 3369.3±156.36 and 3866.3±377.74, respectively. These titers were 4601.2±229.95 and 5110.6±218.30, respectively, in broiler breeder chickens. The variation coefficients in vaccinated and non-vaccinated broiler chickens varied between 46% and 91% in broiler chickens and 29% and 67% in broiler breeder

chickens. These results suggest that antibody titers vary greatly in vaccinated and non-vaccinated animals.

CONCLUSION

The results of this study suggest that ARV infections are very common in the Balıkesir and Bolu provinces, the two main poultry breeding sites in Turkey. The especially high antibody titers detected in broiler breeder chickens used as a resource in poultry breeding suggest that the infection might be present subclinically in this group of animals and vaccination programs have not been regularly administered. Therefore, broiler breeder chickens should be prioritized in programs aimed at combatting the infections. In addition, virological and molecular epidemiological studies to characterize the ARV infections should be conducted to help create and regularly administer vaccination programs.

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Table 2. Results from the chi-square test performed to test for statistical differences between seropositivity rates of vaccinated and non-vaccinated broiler and broiler breeders in Balıkesir and Bolu provinces

Production type	Province		Vaccinated	Non-vaccinated	Total	P		
Broiler	Balıkesir	n	200	138	338	<0.001		
		Positive	156	127	283			
		Negative	44	11	55			
		%	78	92.03	83.72			
	Bolu	n	N/A	178	178		N/A	
		Positive	N/A	139	139			
		Negative	N/A	39	39			
		%	N/A	78.09	78.09			
	BB	Balıkesir	n	100	N/A		100	N/A
			Positive	80	N/A		80	
Negative			20	N/A	20			
%			80	N/A	80			
Bolu		n	100	204	304	0.35		
		Positive	93	183	276			
		Negative	7	21	28			
		%	93	89.71	182.71			
^a Total		Balıkesir	n	300	138	438	<0.001	
			Positive	236	127	363		
	Negative		64	11	75			
	%		78.67	92.03	92.029			
^a Total	Bolu	n	100	382	482	0.03		
		Positive	93	322	415			
		Negative	7	60	67			
		%	93	84.29	93			

Table 3. Results of the chi-square tests performed to test for statistical differences between seropositivity rates in broiler and broiler breeders in Balikesir and Bolu Provinces

Province	Vaccination		Broiler	BB	P	
Balikesir	Vaccinated	n	200	100	0.69	
		Positive	156	80		
		Negative	44	20		
		%	78	80		
	Non-vaccinated	n	138	N/A	N/A	
		Positive	127	N/A		
Negative		11	N/A			
Bolu	Vaccinated	n	N/A	100	N/A	
		Positive	N/A	93		
		Negative	N/A	7		
		%	N/A	93		
	Non-vaccinated	n	178	204	<0.01	
		Positive	139	183		
		Negative	39	21		
		%	78.09	89.71		
	^a Total	Vaccinated	n	200	200	0.03
			Positiv	156	173	
			Negative	44	27	
			%	78	86.5	
^a Total	Non-vaccinated	n	316	204	0.07	
		Positive	266	183		
		Negative	50	21		
		%	84.18	89.71		

Table 4. Mean antibody titers in vaccinated and non-vaccinated broiler and broiler breeder chickens in the Bolu and Balikesir provinces

Production type	Province	Vaccination				P
		Vaccinated		Non-vaccinated		
		n	$\bar{x} \pm S_{\bar{x}}$	n	$\bar{x} \pm S_{\bar{x}}$	
Broilers	Balikesir	200	3369.3±156.36	138	4613.4±830.51	0.083
	Bolu	N/A	N/A	178	3287.0±180.47	N/A
	Total	200	3369.3±156.36	316	3866.3±377.74	0.312
BB	Balikesir	100	3895.2±255.29	N/A	N/A	N/A
	Bolu	100	5307.1±370.61	204	5110.6±218.30	0.628
	Total	200	4601.2±229.95	204	5110.6±218.30	0.109

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