Turk. J. Vet. Anim. Sci.
2008; 32(2): 79-84

Research Article

Investigation of Chicken Infectious Anemia Virus Infection by PCR and ELISA in Chicken Flocks*

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Received: 04.10.2006

Abstract: Presence of the chicken infectious anemia virus (CIAV) infection in chicken flocks in some provinces of Turkey was investigated by ELISA and PCR in this study. From 38 flocks (16 commercial layer, 10 commercial broilers, 4 breeder layers and 8 breeder broilers), 922 serum samples were tested for CIAV-specific antibodies using a commercially available competitive ELISA. CIAV antibodies were positive in 609 (66.0%) sera from 34 flocks (89.5%). A total of 95 thymus samples from 25 flocks, 57 samples from 15 commercial layers, and 38 samples from 10 commercial broiler flocks were tested by PCR. In 53 (55.8%) thymus samples from 20 flocks (80.0%) CIAV-specific DNA was detected. In commercial layers antibodies were detected in 15 (93.7%) of 16 flocks and viral DNA was detected in 13 (86.6%) of 15 flocks. In commercial broilers both CIAV antibodies and DNA were detected in 7 (70%) of 10 flocks tested. While seropositivity was detected in 12 (100%) of breeding broilers (8) and layer (4) flocks tested, no PCR was performed in these flocks. The study showed high prevalence of subclinical CIAV infection in the investigated chicken flocks.

Key Words: Chicken infectious anemia virus, ELISA, PCR, chicken

Tavukçuluk İşletmelerinde Chicken Infectious Anemia Virus Enfeksiyonunun PCR ve ELISA ile Araştırılması

Özet: Bu çalışmada, Türkiye'nin bazı illerinde tavukçuluk işletmelerinde chicken infectious anemia virus (CIAV) enfeksiyonu ELISA ve PCR ile araştırıldı. Toplam 38 kümesten (16 ticari yumurtacı, 10 ticari broyler, 4 damızlık yumurtacı ve 8 damızlık broyler) 922 serum örneği, ticari bir kompetetiv ELISA kiti kullanarak CIAV antikorları yönünden test edildi. Otuz dört işletmeden 609 (% 66,0) serum örneğinde CIAV antikorları pozitif bulundu. Yirmi beş işletmeden 95 timus örneği (15 ticari yumurtacı işletmesinden 57 örnek ve 10 ticari broyler işletmesinden 38 örnek) PCR ile test edildi. Yirmi işletmeden (% 80,0) 53 (% 55,8) timus örneğinde CIAV-spesifik DNA tespit edildi. Ticari yumurtacılarda, 16 işletmeden 15'inde (% 93,7) antikorlar ve 15 işletmeden 13'ünde (% 86,6) DNA belirlendi. Ticari broylerlerde, test edilen 10 işletmeden 7'sinde (% 70,0) hem CIAV antikorları hemde DNA tespit edildi. On iki broyler (8) ve yumurtacı (4) damızlık kümeslerin hepsinde (% 100) sero-pozitiflik belirlenirken, bu kümeslerde PCR yapılmadı. Bu çalışma, araştırılan tavuk işletmelerinde CIAV enfeksiyonunun subklinik olarak yüksek prevalansta olduğunu gösterdi

Anahtar Sözcükler: Chicken infectious anemia virus, ELISA, PCR, tavuk

Introduction

Chicken infectious anemia (CIA) caused by chicken infectious anemia virus (CIAV) is a disease of young chickens (1,2). The causative agent was first isolated by Yuasa et al. (2) in 1978. Since then, the disease has been demonstrated by serological, virus isolation, and PCR methods in various countries (3-12). CIA is characterized by anemia, marked atrophy of bone marrow, thymus, and bursa of Fabricius and severe immunosuppression (13,14). Additionally, specific symptoms are observed such as haemorrhages in leg and chest muscles, focal necrosis in liver, ulcerative erosions in gizzard, and necrosis of wing skin (15).

* This work is a project supported by The Scientific and Technical Research Council of Turkey (TÜBITAK, VHAG-1932).
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CIAV in young chicken causes aplastic anemia, generalised lenfoid depletion and immunosuppression (16). The immunosuppression is responsible for increased mortality, reduced performance and decreased resistance to viral and bacterial diseases in breeding period (17,18). CIA appears mostly in subclinic form (14) and complicated secondarily with viral, bacterial, fungal and parasitic diseases (19). The effect of immunosuppression was subclinically altered with CIAV and Infectious Bursal Disease virus (IBDV), Marek's Disease virus (MDV), Adenovirus (AV), and Reovirus (REV) (13,14,17,20) to increase pathogenicity (19,21).

Diagnosis by virus isolation is time-consuming and requires a well-equipped laboratory and experienced personnel (3,7). However, serology using immunofluorescent antibody (IFA), ELISA, and neutralisation tests can detect antibodies to CIAV (6,8,9). Indirect diagnosis, using serological tests solely, is not reliable because of some disadvantages of these tests. Thus, serological data needs to be evaluated in the light of other diagnostic tests (9,11). Because PCR is rapid, sensitive, specific, and gives an opportunity to diagnose diseases using tiny amount of tissue or biological liquid samples, it could be used to monitor flock diseases (4,5,7,12,22,23).

The aim of this study was to detect CIAV infection, together with other infections under field conditions, by ELISA and PCR in Turkey.

Materials and Methods

Serum Samples

From 38 flocks with different age of chickens located in several provinces of Turkey, 922 blood samples were collected (Table 1). These flocks were 16 commercial layer (4-36 weeks of age), 10 commercial broiler (11-39 days of age), 4 breeder layer (20-42 weeks of age), and 8 breeder broiler breeding (30-63 weeks of age). From each flock, 20 to 25 serum samples were taken. None of the flocks has had a history of vaccination against CIAV.

Table 1. History of the tested flocks.

Layers				Broilers			
Flock number	Location	Age (week)	Other Infections	Flock number	Location	Age (day)	Other Infections*
1	Afyonkarahisar	8	-	1	Afyonkarahisar	15	Salmonellosis
2	Afyonkarahisar	12	-	2	Afyonkarahisar	36	-
3	Afyonkarahisar	18	Salmonellosis	3	Konya	22	-
4	Afyonkarahisar	16	IBD	4	Konya	11	E. coli
5	Afyonkarahisar	36	Leucosis	5	Karaman	21	-
6	Konya	4	IBD	6	Ankara	24	-
7	Konya	12	IBD	7	Konya	39	IBD
8	Konya	16	IBD	8	Ankara	31	-
9	Elazığ	23	-	9	Konya	34	IBD
10	Konya	10	MD	10	Karaman	25	-
11	Konya	9	MD				
12	Konya	10	MD, E. coli				
13	Çorum	21	MD				
14	Çorum	10	MD, E. coli				
15	Konya	9	IBD				
16	Bursa	One day.	-				

* The data is based on the records taken by the veterinary on duty. Before samples were taken, diagnosis of a veterinarian and/or the laboratory of poultry diseases were stated.

Tissue Samples

A total of 95 thymus samples for amplification of CIAV DNA were collected from 15 commercial layer (57 samples) and 10 broiler flocks (38 samples). However, sampling for thymus could not be performed from a flock (number 16 of flock).

ELISA

Sera were tested for CIAV-specific antibodies using a commercial competitive ELISA Kit (Chicken Infectious Anemia Test Kit, IDEXX Laboratories, Inc., Westbrook, Maine 04092, USA). Sera were diluted 1/10 and tested according to the manufacturer's instructions.

PCR Assay

DNA Extraction: CIAV DNA was extracted from thymus samples of 25 mg, using a commercial kit (DNeasy Blood&Tissue Kits, Qiagen Ltd., Qiagen House, Fleming Way, Crawley, West Sussex, RH 10 9NQ). DNA extraction processes were made according to manufacturer's instructions.

Primers: PCR was performed using primers specific for ORF3, which coded VP3 and produced a band of 298 bp (12). They were purchased from Genosys Biotecnologies (Cambridge, UK).

5' ACG CTC TCC AAG AAG ATA CTC CAC CC-3'

5' TTT AGC TCG CTT ACC CTG TAC TCG GAG G-3'

DNA Amplification: The PCR assay was carried out in a final volume of 50 ml mixture consisted of PCR buffer (10 mM Tris-HCl (pH 8.3)), 50 mM KCl, 0.001% gelatin and 1.5 mM $MgCl_2$ (Sigma), 200 µM each of the deoxynucleoside triphosphates, 1 mM each of the primers, 1.25 U *Taq* DNA polymerase (Sigma), and 2 µL template.

The amplification was performed under the following conditions in a thermal cycler (MJ Research, Inc., Watertown, MA, USA): a denaturation step of 94 ºC for 3 min followed by 35 cycles of 94 ºC for 1 min, 59 ºC for 1 min, 72 ºC for 2 min, with a final extension at 72 ºC for 5 min. The PCR product was then analysed by electrophoresis in 1.5% agarose gel and visualised under ultraviolet light after staining with ethidium bromide (12).

Results

ELISA Results. Out of 922 samples from a total of 38 flocks, 609 (66.0%) were found positive while 313 (33.9%) were observed negative by ELISA (Table 2). In 16 commercial layer flocks, 278 (70.9%) were positive out of 392 serum samples. When evaluating flocks, 15 of 16 flocks (93.7%) were positive. In commercial broiler flocks (n=10), out of 240 sera 50 (20.8%) were positive and 190 (79.2%) were negative. In addition, 7 broiler flocks (70.0%) were positive. Analysis of 90 samples taken from 4 layer breeding flocks showed that all had 100% positive results. Out of 200 sera, obtained from 8 broiler breeding flocks, 191 (95.5%) were positive and 9 (4.5%) negative. On the other hand, all broiler-breeding flocks (100%) were determined positive.

PCR Results. Bands with the weight of 298 bp were evaluated as positive (Figure). PCR analysis revealed that

Table 2. ELISA results of chicken sera.

Flock type	Number of tested		Number of flocks				Number of sera			
	Flocks	Sera	Positive		Negative		Positive		Negative	
			n	%	n	%	n	%	n	%
Commercial layer	16	392	15	93.7	1	6.3	278	70.9	114	29.1
Commercial broiler	10	240	7	70.0	3	30	50	20.8	190	79.2
Broiler breeding	8	200	8	100	-	0	191	95.5	9	4.5
Layer breeding	4	90	4	100	-	0	90	100	0	0
Total	38	922	34	89.5	4	10.5	609	66.0	313	33.9

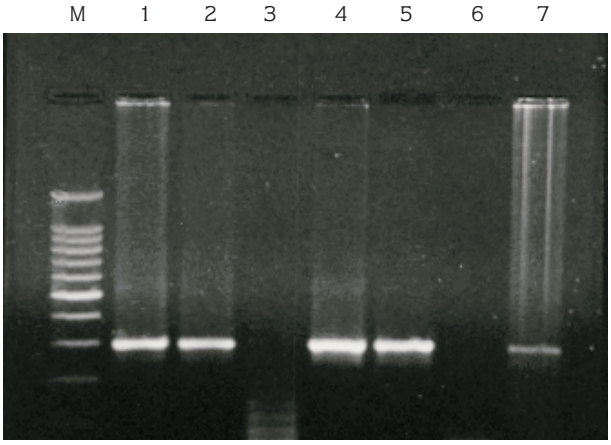

Figure. Results of PCR analysis of thymus samples: M: 100 bp DNA Marker; 1, 2, 4, 5, 7: Positive samples (298 bp band); 3, 4: Negative samples.

53 (55.8%) of 95 samples from a total of 25 flocks (15 commercial laying and 10 commercial broiler flocks) were positive (Table 3). While 38 (66.7%) of 57 thymus samples were positive taken from 15 commercial layer flocks and 15 (39.5%) of 38 thymus samples taken from 10 commercial broiler flocks were positive (Table 3).

Discussion

CIA is a very common infection taking a part in the aetiology of some multifactorial diseases in layer and broiler flocks of both commercial and breeding types throughout the world (18,24). One of the most outstanding features of the CIAV is to cause immunosuppression by itself directly or by participating indirectly with other viruses in chickens (13,19,21,24,25).

Some serological (Neutralisation, ELISA, and IFAT) (6,8,9,26) and molecular studies (PCR) (7,12,23,27) on determining either presence of antibodies to the agent or amplification of causatives' DNA have been reported. Ergün et al. (8) examined 4 different broiler flocks by ELISA and observed that 68.2% of the flocks were positive. Positive results were also reported in 4 (27.7–100%) of 10 parent stocks by ELISA. Kuyucuoğlu et al. (26) reported that a high degree of positivity (85.7%) was also found by ELISA from a total of 21 commercial layer flocks. It was also stated that CIAV antibodies were detected in 66.0% of commercial broiler and layer, broiler-breeding of flocks by ELISA in provinces İzmir and Bandırma (28). By virus neutralization test, more than 89% of the flocks (either commercial broiler or layer commercial or layer parent) were found to be positive (6). Finally, CIA is one of the common infections according to serologic results in commercial flocks in Turkey. This study is in line with previous studies.

Some researchers (9,29) reported positive levels of antibodies to CIAV by IFA in more than 89.5% of broilers or layers (both type of breeding) in the USA. These and other studies from all over the world showed that the infection is common in all chicken types (8,10,11,26,28-30).

PCR, used widely due to some difficulties in the isolation of virus (5,7,12), gives the flexibility to researchers for both in vivo and in vitro studies (30) and an opportunity to select right strain of virus for vaccine production (4). Additionally, CIAV contaminations in cell cultures or various vaccines can be demonstrated by PCR (5,23). Thymus is the most suitable organ for determining CIAV's DNA by PCR (4).

Table 3. PCR results in thymus samples.

Flock type	Number of tested		Number of flocks				Number of thymus samples			
	Flocks	Thymus	Positive		Negative		Positive		Negative	
			n	%	n	%	n	%	n	%
Commercial layer	15	57	13	86.6	2	13.4	38	66.7	19	33.3
Commercial broiler	10	38	7	70.0	3	30.0	15	39.5	23	60.5
Total	25	95	20	80.0	5	20.0	53	55.8	42	44.2

Yılmaz et al. (12) investigated the presence of virus DNA in limited numbers of thymus samples from broilers in 8 different flocks and reported that the lowest occurrence (8.5%) was in Marmara Region in Turkey. On the other hand, this study is more comprehensive because CIA positive chickens were detected using PCR and ELISA. In the current study, the number of anti-CIAV antibody positive chickens from any type of breeding is actually much higher than those reported by Yılmaz et al. (12).

CIAV is known to act synergically with IBDV and Marek (17,19,25). When the history of flocks evaluated, we found out that some of the CIAV positive flocks had also showed other infections (IBD, MD, and Leucosis). Thus, which infection within the flock triggered by which infection is not clear (17,19,25). Not only viral but also some bacterial infections like *Salmonella* sp. and *E. coli* in commercial chicken flocks were also reported. When the ages of the animals were taken into account from commercial layers and broilers, the occurrence of CIA with IBD or some bacterial infections may be explained by a possible immunosuppression caused by CIAV prior to IBD or bacterial infection.

In conclusion, the results of our study showed that CIAV infection in Turkey is more common than expected in poultry. Therefore, CIAV infected positive chicken flocks may have already been infected with other causative agents and we strongly recommend using vaccines to CIA in a vaccination schedule in parent stocks countrywide. Vaccination may prevent further virus spread and can also minimize vertical transmission of virus in field conditions.

Acknowledgment

This work was granted by The Scientific and Technological Research Council of Turkey (TÜBİTAK) (Project no. VHAG-1932)

References

1. Barbour, E.K., Farran, M.T., Hamadeh, S.K., Bouljihad, M., Faroon, O., Kreydiyyeh, S.: Comparative impact of live chicken infectious anemia virus vaccine versus natural exposure in meat chicken breeders on immunity to infectivity by CIA and inclusion body hepatitis viruses in their offspring. Vet. Res. Commun. 2002; 26: 397-405.
2. Yuasa, N., Taniguchi, T., Yoshida, I.: Isolation and some characteristics of an agent inducing anemia in chics. Avian Dis., 1979; 23: 366-385.
3. Brentano, L., Mores, N., Wentz, I., Chandratilleke, D., Schat, K.A.: Isolation and identification of chicken infectious anemia virus in Brazil. Avian Dis., 1991; 35: 793-800.
4. Todd, D., Mawhinney, K.A., McNulty, M.S.: Detection and differentiation of chicken anemia virus isolates by using the polymerase chain reaction. J. Clin. Microbiol., 1992; 30: 1661-1666.
5. Soiné, C., Watson, S.K., Rybicki, E., Lucio, B., Nordgren, R.M., Parrish, C.R., Schat, K.A.: Determination of the detection limit of the polymerase chain reaction for chicken infectious anemia virus. Avian Dis., 1993; 37: 467-476.
6. Drén, C., Farkas, T., Németh, I.: Serological survey on the prevalence of chicken anemia virus infection in Hungarian chicken flocks. Vet. Microbiol., 1996; 50: 7-16.
7. Zhou, W., Shen, B., Yang, B., Han, S., Wei, L., Xiao, B., Zhou, V.: Isolation and identification of chicken infectious anemia virus in China. Avian Dis., 1997; 41: 361-364.
8. Ergün, A., Yurtman, M., Nalbantsoy, A.: Civciv anemisi hastalığı (CAV) üzerinde ELISA testi ile sero-sörvey çalışma. Çiftlik Derg., 1998; 5: 44-46.
9. Cardona, C., Lucio, B., O'Connell, P., Jagne, J., Schat, K.A.: Humoral immune responses to chicken infectious anemia virus in three strains of chickens in a closed flock. Avian Dis., 2000; 44: 661-667.
10. De Herdt, P., Van den Bosch, G., Ducatelle, R., Uyttebroek, E., Schrier, C.: Epidemiology and significance of chicken infectious anemia virus infections in broilers and broiler parents under nonvaccinated European circumstances. Avian Dis., 2001; 45: 706-708.
11. Ledesma, N., Fehervari, T., Casaubon, M.T., Lucio, E., Ratz, F.: Chicken infectious anemia in Mexico: virus identication and serology survey. Avian Dis., 2001; 45: 788-796.
12. Yılmaz, H., Turan, N., Özgür, N. ., Helps, C.R., Akay, Ö.: Detection of chicken anemia virus DNA in the thymus of naturally infected chicks in Turkey. Avian Dis., 2001; 45: 529-533.
13. Cloud, S.S., Rosenberger, J.K., Lillehoj, H.S.: Immune dysfunction following infection with chicken anemia agent and infectious bursal disease virus. II. Alteriations of in vitro lymphoproliferation and in vivo immune responses. Vet. Immunol Immunopathol., 1992; 34: 353-366.
14. McConnell, C.D.G., Adair, B.M., McNulty, M.S.: Effects of chicken anemia virus on cell-mediated immune function in chickens exposed to the virus by a natural route. Avian Dis., 1993; 37: 366-374.

15. Smyth, J.A., Moffett, D.A., McNulty, M.S., Todd, D., Mackie, D.P.: A sequantial histopathologic and immunocytochemical study of chicken anemia virus infection at one day of age. Avian Dis., 1993; 37: 324-338.

16. Toro, H., Ramirez, A.M., Larenas, J.: Pathogenicity of chicken anemia virus (isolate 10343) for young and older chickens. Avian Pathol., 1997; 26: 485-499.

17. Rosenberger, J.K., Cloud, S.S.: The effects of age, route of exposure, and coinfection with infectious bursal disease virus on the pathogenicity and transmissibility of chicken anemia agent (CAA). Avian Dis., 1989; 33: 753-759.

18. McIlroy, S.G., McNulty, M.S., Bruce, D.W., Smyth, J.A., Goodall, E.A., Alcorn, M.J.: Economic effects of clinical chicken anemia agent infection on profitable broiler production. Avian Dis., 1992; 36: 566-574.

19. Otaki, Y., Nunoya, T., Tajima, M., Saito, K., Nomura, Y.: Enhanced pathogenicity of chicken anemia agent by infectious bursal disease virus relative to the occurence of Marek's disease vaccination breaks. Jpn. J. Vet. Sci., 1989; 51: 849-852.

20. Jørgensen, P.H., Otte, L., Nielsen, O.L., Bisgaard, M.: Influence of subclinical virus infections and other factors on broiler flock performance. Br. Poult. Sci., 1995; 36: 455-463.

21. Toro, H., Gonzalez, C., Cerda, L., Hess, M., Reyes, E., Geissea, C.: Chicken anemia virus and fowl adenoviruses: association to induce the inclusion body hepatitis/hydropericardium syndrome. Avian Dis., 2000; 44: 51-58.

22. Tham, K.M., Stanislawek, W.L.: Polymerase chain reaction amplification for direct detection of chicken anemia virus-DNA in tissues and sera. Avian Dis., 1992; 36: 1000-1006.

23. Markowski-Grimsrud, C.J., Miller, M.M. , Schat, K.A.: Development of strain-spesific real-time PCR and RT-PCR assays for quantitation of chicken anemia virus. J. Virol. Methods, 2002; 101: 135-147.

24. Hagood, L.T., Kelly, T.F., Wright, J.C., Hoerr, F.J.: Evaluation of chicken infectious anemia virus and associated risk factors with disease and production losses in broilers. Avian Dis., 2000; 44: 803-808.

25. Miles, A.M., Reddy, S.M., Morgan, R.W.: Coinfection of spesific-pathogen-free chickens with Marek's disease virus (MDV) and chicken infectious anemia virus: effect of MDV pathotype. Avian Dis., 2001; 45: 9-18.

26. Kuyucuoğlu, Y., Hadimli, H.H., Kenar, B., Uçan, U.S.: Detection of chicken infectious anemia virus antibody in layer operations by using ELISA in Afyon region. Vet. Hek. Mikrobiyol. Derg., 2003; 3: 21-26.

27. Noteborn, M.H.M., Koch, G.: Chicken anemia virus infection: molecular basis of patogenicity. Avian Pathol., 1995; 24: 11-31.

28. Türkyılmaz, S., Mısırlıoğlu, O.Z.: İzmir ve Bandırma yörelerinde chicken infectious anemia virus antikorlarının ELISA ile saptanması. Pendik Vet. Mikrobiyol. Derg., 2004; 35: 13-19.

29. Lucio, B., Schat, K.A., Shivaprasad, H.L.: Identification of the chicken anemia agent, reproduction of the disease, and serological survey in the United States. Avian Dis., 1990; 34: 146-153.

30. Yamaguchi, S., Kaji, N., Munang'andu, H.M., Kojima, C., Mase, M., Tsukamoto, K.: Quantification of chicken anemia virus by competitive polymerase chain reaction. Avian Pathol., 2000; 29: 305-310.