

# DETECTION OF ANTIBODY TO CHICKEN ANAEMIA VIRUS USING ELISA TEST IN INDONESIA

## PELACAKAN ANTIBODI TERHADAP VIRUS PENYEBAB ANEMIA UNGGAS MENGUNAKAN UJI ELISA DI INDONESIA

Ida Lestari Soedijar<sup>1</sup>, Marthen B Malole<sup>2</sup>, and Baharuddin Syahroni<sup>1</sup>

<sup>1</sup>Balai Pengujian Mutu dan Sertifikasi Obat Hewan (BPMSOH) Departemen Pertanian RI,  
Gunungsindur-Serpong, INDONESIA, Telp. (62-21) 7560489

<sup>2</sup>Laboratorium Virologi Bagian Penyakit Hewan dan Kesehatan Masyarakat Veteriner Fakultas Kedokteran Hewan  
Institut Pertanian Bogor, Jl. Taman Kencana 3, Bogor 16151, INDONESIA, Telp. / Faks. (62-251) 381436

### ABSTRACT

*Media Veteriner. 1999. 6(3): 11-13.*

Serological study concerning the presence of antibody to CAV was carried out by implementing ELISA test to 148 samples of sera collected from native and improved breed (layer and broiler) from several areas of Indonesia namely Medan (North Sumatera), Bogor, Tangerang and Bekasi (West Java), Manado (North Sulawesi). Ujung Pandang (South Sulawesi) during May 1995. The results indicated that antibody to CAV were detected in all study area at quite high titer both in native and improved chickens at prevalence rate of 20-83% and 10-90%, respectively. It is suggested that CAV infection is widely spread in Indonesia and further study need to be carried out to characterize the virus infecting those chickens.

**Key words:** Chicken Anaemia Virus, ELISA, Indonesia

### ABSTRAK

*Media Veteriner. 1999. 6(3): 11-13.*

Keberadaan antibodi virus CAV telah diteliti memakai metode ELISA, menggunakan 148 contoh sera dari ayam buras dan ras (petelur dan pedaging) dari beberapa wilayah di Indonesia yaitu Medan (Sumatera Utara), Bogor, Tangerang dan Bekasi (Jawa Barat), Manado (Sulawesi Utara), dan Ujung Pandang (Sulawesi Selatan) selama bulan Mei 1995. Dari hasil penelitian diperoleh indikasi bahwa antibodi CAV dapat dideteksi pada semua wilayah dengan titer yang cukup tinggi dan prevalensi 20-83% pada ayam buras dan 10-90% pada ayam ras. Diasumsikan bahwa infeksi CAV tersebar luas di Indonesia maka diperlukan penelitian lebih lanjut untuk memperoleh karakter virus yang menginfeksi ayam tersebut.

**Kata-kata kunci:** Chicken Anaemia Virus, ELISA, Indonesia

### INTRODUCTION

Chicken Anaemia Virus (CAV, Gifu-1 strain) was first isolated in Japan in 1979 by Yuasa (Wicht, 1993), causing anemia in antibody-free 1-day-old chick. Since then numerous publications have documented the world wide occurrence of CAV in chickens (Engstrom, 1988; Vielitz and Landgraf, 1988; Rosenberger and Cloud, 1989; Otaki *et al.*, 1991; Cloud *et al.*, 1992; Goodal and Alcorn, 1992).

Chicken anaemia virus is a single stranded DNA virus but its taxonomic classification has not been totally resolved (McNulty *et al.*; 1990, McNulty *et al.*, 1990). Clinical signs of the diseases include depressed weight gain, paleness, clotting abnormalities, lymphocytopenia and anemia (Chettle *et al.*, 1989).

The diseases is characterized by aplastic anemia and generalized lymphoid atrophy with a concomitant immunosuppression. Consequently, infectious anemia is frequently complicated by secondary viral, bacterial or fungal infections. Growth is retarded and mortality is generally between 10%-20% (Cloud *et al.*, 1992; Aboulela, 1998).

When the cost of therapy and carcass downgrading were taken into account, affected chicken had a net income of about 18% (Goodwin *et al.*, 1992) to 25% (Aboulela, 1998) lower than that of normal flock.

The diseases afflicts young broiler chicks between one and three weeks of age (Engstrom, 1988; Goodwin *et al.*, 1989; Rosenberger and Cloud. 1989). In Indonesia, antibody against CAV was detected in several chicken farms in Bogor in 1995, when the serum were tested at Intervet Laboratory Netherlands (Soedijar, unpublished data). Since many veterinarian of Indonesian technical services suspected the occurrence of CAV infection in the field, this study was designed to obtain more information on the prevalence of CAV antibody using ELISA.



## MATERIALS AND METHODS

### Serum Sampling

Sera were collected from chickens in age of 11 days to 3 years consisted of a total of 88 native and 60 improved chickens (layer and broiler) obtained from several farms in Indonesia namely Medan (North Sumatera), Bogor, Tangerang and Bekasi (West Java), Manado (North Sulawesi), Ujung Pandang (South Sulawesi) during May 1995 (Table 1). All the chickens have had only ND or IBD vaccination experience.

### Enzyme-linked Immunosorbent Assay (ELISA)

Commercially available ELISA kits (Guildhay Ltd., Guildford Surrey, England) to assay samples for antibodies to CAV were used. Positive sera, which were kindly supplied by Dr. K. Taguchi from National Veterinary Assay Laboratory Japan, were also used as positive controls. The method used was followed the recommendation from the company. Briefly, aliquot of 50 microlitres of 1:500 sera dilution from both positive and tested sera were inoculated into the kit, and the plate was incubated for 1 hour at 37 °C, washed four times with washing buffer. Then 50 microlitres of enzyme conjugate (alkaline phosphatase labelled donkey anti chicken IgG), were added and the plate was incubated again for 1 hour at 37 °C. The plates were washed 4 times (300 microliters per well) again. Fifty micro liters of substrate (phenolphthalein monophosphate) were added and the plate was reincubated for 30 minutes at 37 °C. Colour develop-

ment was light pink which deepened on addition of stop solution (sodium hydroxide). The plates were read at 550 nm, using a microtitre/ELISA plate reader, and the titers were calculated by following formula:

$$S/P = \frac{\text{Sample Absorbance} - \text{Negative Control Absorbance}}{\text{Positive Control Absorbance} - \text{Negative Control Absorbance}}$$

$$\text{Sample Titer} = \text{antilog of } \{ \log (S/P) \times 1.24 + 3.70 \}$$

For the test to be valid, the mean absorbance of negative control and positive control sera should be less than 0.2 and greater than 0.6 respectively. Serum samples were considered negative if their titer was less than and were interpreted as positive if the titer were higher than 900. Readings between 280 and 900 were classified as dubious.

## RESULTS AND DISCUSSIONS

Data obtained from this study as presented in Table 1 indicated that antibody to CAV were detected in sera collected from all the study areas. Assuming that antibody is one of the indicator of the presence of infections, this study revealed that the infection of CAV is widely spread in Indonesia affecting both native and improved breed of chicken at different age. The prevalence rate would have been

Table 1. The result of sera from layer/broiler and native chickens in each area

Region	Chicken	Age (weeks)	E L I S A		Positive (%)	Vaccination experience
			Negative	Positive		
Manado	Native	4-10	10 (0-185)	----	50	ND <sup>a</sup>
		12-36	----	10 (2958-5648)		
Ujung pandang	Native	15	18 (0-335)	2 (1143,1216)	10	ND, IBD <sup>b</sup>
		40	3 (0-293)	----	83	ND
Medan	Native	156	----	15 (2000-6172)		
		72	1 (136)	9 (1300-4843)	90	ND
Bogor	Native	4	6 (0-293)	----	70	ND
		29	----	10 (2112-6936)		
		44	----	2 (1478,4250)		
Tangerang	Native	96	----	2 (1555,2318)		
		6	1 (672)	1 (1671)	70	--
		8	4 (0-279)	8 (1949-6147)		--
		12	1 (0)	3 (2465-4795)		--
Bekasi	Broiler	20	----	1 (6147)		--
		24	----	1 (3024)		--
		4	8 (0-453)	2 (1308,1402)	20	ND
		12	1 (326)	1 (5797)	90	ND, IBD
Bekasi	Broiler	16	----	2 (5109,5451)		
		20	----	6 (2050-6527)		
		2	10 (0-842)	10 (929-3267)	50	ND

<sup>a</sup>Newcastle Disease Vaccination; <sup>b</sup>Infectious Bursal Disease Vaccination



higher than what was obtained from this study if virus neutralization (VN) was used as it is more sensitive than ELISA (Noteborn *et al.*, 1991). Native chicken become infected, however there was no report of clinical signs related to CAV infection in this country.

This study also pointed out that chickens older than 6 weeks showing increasing degree of prevalence rate and antibody titer, indicating that the infection is persisting in the population of native as well as improved breed of chickens. Consequently, young chickens got maternal antibody that persisted until 4 weeks as indicated by the decreasing of the prevalence rate and antibody titer from 2 week-old-broiler (Bekasi) as compared to 4 week-old-native chicken (Tangerang, Medan, Manado), This analysis is in line with the study reported by Pagés-Manté *et al.*, (1997) in which progeny of vaccinated breeder showed sufficient level of maternally derived antibody to CAV for up to 3 to 4 weeks.

### CONCLUSION

Antibody to CAV as measured by ELISA was present in sera collected in north Sumatera, West Java, North Sulawesi and South Sulawesi at variable percentage namely 20-83% of native and 10-90% of improved breed of chicken. These data lead to the conclusion that CAV infection is widespread in these areas of Indonesia. This preliminary result will help further investigator to be aware of the threatening of CAV in poultry industry, particularly of its immunosuppressive effect.

### ACKNOWLEDGEMENT

The authors are greatly indebted to Dr. K. Taguchi (National Veterinary Assay Laboratory, Japan) for supporting positive serum and his cooperation. Also, we wish to thank Mr. Hanz (Akzo Nobel, Intervet, Holland) for his support with ELISA kits.

### REFERENCES

- Aboulela, M. A. 1998. CAV and Broiler Downgrading. *Poultry International*, 37(4): 30-31.
- Chettle, N.J., R.K. Eddy, P.J. Wyeth, and S.A. Lister. 1989. An Outbreak Disease Due to Chicken Anaemia Agent and in Broiler Chicken in England. *Vet. Rec.*, 124: 211-215.
- Cloud, S.S., J.K. Rosenberger and S.A. Lister. 1992. Immune Dysfunction Following Infection Bursal Disease Virus II. Alterations of *in vitro* Lymphoproliferation and *in vivo* Immune Responses. *Vet. Immun. Immun.*, 34: 353-366.
- Engstrom, B.E. 1988. Blue Wing Diseases of Chicken Anaemia Agent. *Avian Pathology*, 17: 23-33.
- Goodwin, M.A, J. Brown, S.L. Miller, M.A. Smeltzer, W.L. Steffens and W.D. Waltman. 1989. Infectious Anaemia Caused by a Parvovirus-like Virus in Georgia Broiler. *Avian Diseases*, 33: 438-445.
- Goodwin, M.A, J. Brown, and T.G. Dickson. 1992. Increasing Incidence of Anaemia Among Clinically Ill Georgia Brpiler. 1998-1989 vs 1990. *Avian Diseases*, 36: 1019-1021.
- Goodal, E.A. and M.J. Alcorn. 1992. Economic Effects of Clinical Chicken Anaemia Agent Infection on Profitable Broiler Production. *Avian Diseases*, 36: 566-574.
- McNulty, M.S., T.J. Connor, F. McNeilly, M.F. McLoughlin, and K.S. Kirkpatrick. 1990. Preliminary Characterization of Isolates of Chicken Anaemia Agent from the United Kingdom. *Avian Pathology*, 19: 67-73.
- McNulty, M.S., W.L. Curran., D. Todd and D.P. Mackie. 1990. Chicken Anaemia Agent and Electron Microscopic Study. *Avian Diseases*, 34: 736-743.
- Noteborn, M.N., G.F. de Bour., D.J. Roozelaar., C. Karreman., O. Kranenburg., J.G. Vos., S.H.M. Jeurissen., R.C. Hoeben., A. Zantema., G. Koch., H. Ormond., and A.J. Eb. 1991. Characterization Cloned Chicken Anaemia Virus DNA that Contain All Elements for the Infectious Diseases Replication Cycle. *J. of Virology*, 65: 3131-3139.
- Otaki, Y., K. Saito., M. Tajima and Y. Nomura. 1991. Detection of Antibody Chicken Anaemia Agent. A Comparison of Their Serological Test. *Avian Pathology*, 20: 315-324.
- Pagés-Manté A., N. Saubi, C. Artigas, and E. Espuna. 1997. Experimental Evaluation of an Inactivated Vaccine against Chicken Anaemia Virus. *Avian Pathology*, 26(4): 721-729.
- Rosenberger, J.K. and S.S. Cloud. 1989. The Isolation and Characterization of Chicken Anaemia Agent (CAA) from Broiler in the United States. *Avian Diseases*, 33: 707-713.
- Vielitz, E. and H. Landgraf. 1988. Anaemia Dermatitis of Broiler: Field Observation on its Occurrence, Transmission and Prevention. *Avian Pathology*, 17: 113-120.
- Wicht, J.V. 1993. Chicken Anaemia Agent in South Africa. *Vet. Rec.*, (133): 147-148.