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DETECTION OF ANTIBODIES TO *Toxoplasma gondii* IN CHICKEN BY AVIDIN-BIOTIN ELISA*

K. Devada and R. Anandan*****

Department of Parasitology
Madras Veterinary College, Chennai - 7

The specificity and sensitivit of enzyme immunoassays can be enhanced by the use of immuno-reactants or co-factors of low molecular weight coupled to an antigen or antibody. The co-factors commonly used for this purpose include avidin (mol. wt. - 68000) and biotin (mol.wt. - 244). The antibodies labelled with biotin are subsequently linked to immunoenzymes like horse radish peroxidase through avidin, bringing about magnification and amplification at the level of co-factor-enzyme interaction. Guesdon *et al.*(1979) were the first to incorporate avidin-biotin interaction to ELISA technique and found it satisfactory in amplifying ELISA. Kendall *et al.* (1983), Subba Rao *et al.* (1983), Ramesh Babu and Rajasekharan (1988), Vivek Kapur (1989), Prashant (1992) and Thimma Reddy *et al.* (1994), also found the modified technique more sensitive and useful. Since chicken is considered to be clinically tolerant and evince poor response to toxoplasmosis (Biancifiori *et al.*, 1986), an improved and more specific and sensitive technique than ELISA was employed for the estimation of *T. gondii* antibodies in chicken using tachyzoite antigen.

Materials and Methods

Five hundred serum samples collected from birds brought to the slaughter houses at Parrys and Pudupet markets of Madras City and to the Veterinary College Hostel, formed the test sera. This consisted of 376 samples from exotic birds and 124 from local breeds. The sera were separated under aseptic conditions and stored at -20°C until further use.

Toxoplasma gondii tachyzoite antigen prepared as outlined by Dubey *et al.* (1993) was used in the present assay.

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** *Present address: Assistant Professor, Department of Parasitology, College of Vety. and Animal Sciences, Mannuthy, Kerala*

*** *Professor and Head (Retd.). Department of Parasitology, MVC, Chennai-7*

Sera of the experimentally infected chicken and those procured from different sources which showed high absorbance values in the ELISA formed the positive control sera. The negative control sera were collected from healthy birds which were non-infected and kept along the experimental group.

The main constituents of this assay like avidin conjugated horse-radish peroxidase (Avidin HRP), biotin and anti-chicken IgG were procured from M/s. Sigma Chemicals. The biotinylation of rabbit antichicken IgG was carried out as per the method recommended by Subba Rao *et al.* (1983).

The optimum concentrations of the *T. gondii* antigen, test sera, avidin HRP and biotin IgG were standardised by the checker board titration. The optimum dilution which gave the highest titre with positive serum and the lowest titre with the negative serum was selected as the working dilution. The optimum dilutions of the *T. gondii* antigen, test sera, avidin HRP and biotin-IgG used in the present work were 1 in 200, 1 in 50 to 1 in 1600, 1 in 600 and 1 in 800 respectively. Negative control sera were diluted 50 times.

The methodology described by Ramesh Babu (1986) was followed. The assay was done in polysterene 96-well flat bottommed micro titre plates (Corning). The absorbance values were recorded at 405 nm in a multiscan ELISA reader. The highest dilution of the test serum which showed double the absorbance value of the average of that of the negative sera was taken as the antibody titre of that particular sample.

Results and Discussion

The results of seropositive chicken in total and at each serial dilution are furnished in Tables 1 and 2 respectively.

Table 1. Seropositivity of chicken sera against *Toxoplasma gondii* by AB-ELISA

Chicken	Number examined	Number positive	Percent positive
Exotic	376	151	40.16
Country	124	72	58.06
Total	500	223	44.60

Table 2. Antibody titres in chicken sera against *Toxoplasma gondii* by AB-ELISA

Chicken	Number examined	Reciprocal titre											
		100		200		400		800		1600		3200	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Exotic	376	53	14.10	45	11.97	26	6.91	16	4.26	11	2.92	-	-
Country	124	42	33.87	27	21.77	2	1.61	-	-	1	0.81	-	-
Total	500	95	19.00	72	14.40	28	5.60	16	3.20	12	2.40	-	-

Among 500 chicken sera screened for *T. gondii* antibodies by AB-ELISA, 223 samples (44.60%) were found to be sero-positive. Out of 376 exotic and 124 country birds, 151 samples (40.16%) and 72 samples (58.06%) respectively were detected to possess antibodies.

Two thirds of the positive samples had lower titres. The maximum titre obtained in the present study was 1600. It was noted that a higher per cent of country birds revealed antibodies than the exotic birds at dilutions of 1 in 100 (33.87%) and 1 in 200 (21.77%).

The higher rate of infection in country birds, than in the exotic birds indicates the eating habits of the former. Since birds are asymptomatic carriers, the present study confirms the utility of serological tests in the detection of *T. gondii* antibodies. The sensitivity and specificity of this test amplified by the use of avidin and biotin confirm the findings of the earlier workers like Kendall *et al.* (1983), Ramesh Babu and Rajasekharan (1988) and Thimma Reddy *et al.* (1994).

Summary

Avidin-biotin ELISA technique was standardised and applied to measure the antibody levels in chicken against toxoplasmosis. Among 500 chicken sera screened, 223 samples (44.60%) were found to be seropositive, in which 151 (40.16%) were from exotic birds and 72 (58.06%) from country birds. Most of the sera had low titres of *T. gondii* antibodies.

References

Biancifiori, F., Rodini, C., Grelloni, V. and Frescura, I. (1986). Avian toxoplasmosis : Experimental infection of chicken and pigeons. *Comp. Immuno. Microbiol. infect. Dis.* **9**: 337-346

Dubey, J.P., Ruff, M.D., Camargo, M.E., Shen, S.K., Wilkins, G.L., Kwok, O.C.H. and Thulliez, P. (1993). Serologic and parasitologic responses of domestic chickens after oral inoculation with *Toxoplasma gondii* oocysts. *Am. J. Vet. Res.* **54**: 1668-1672

Guesdon, J., Terhynck, T. and Avermeas, J. (1979). The use of avidin biotin interaction in immunoenzymatic techniques. *J. Histochem. Cytochem.* **27**: 1131-1139

Kendall, C., Ionescu - Matin, I. and Dreeman, G.R. (1983). Utilisation of biotin-avidin system to amplify the sensitivity of the enzyme linked immunosorbent assay. *J. Immunol. Meth.* **56** 329-339

Prashant, C.S. (1992). Passive transfer and persistence of *Theileria annulata* vaccinated cattle antibodies in calves by avidin biotin *ELISA* test. *M.V.Sc. thesis* submitted to the University of Agricultural Sciences, Bangalore

Ramesh Babu, N.G.(1986). Estimation of Rinderpest antibodies in sheep and goat by an avidin-biotin micro enzyme linked immunosorbent assay. *M.V.Sc. thesis* submitted to the University of Agricultural Sciences, Bangalore

Ramesh Babu, N.G. and Rajasekharan, M. (1988). Prevalence of rinderpest antibodies in sheep and goats in southern India. *Vet. Rec.* **123**: 595-597

Subba Rao, P., Mc Cartney-Francis, N.L. and Metcalfe, D.D. (1983). An avidin-biotin micro *ELISA* for rapid measurement of total and allergen specific human IgE. *J. Immunol. Meth.* **57**: 71-85

Thimma Reddy, P.M., Jaganath, M.S. and Rajasekharan, M. (1994). Assay of *Theileria annulata* antibodies in vaccinated cattle by avidin biotin *ELISA*. *Indian Vet.J.* **71**: 763-767

Vivek Kapur (1989). Transmission and persistence of maternally derived antibodies in New Castle disease virus in broiler chicks. *M.V.Sc. thesis* submitted to the University of Agricultural Sciences, Bangalore