ANTIGENIC SIMILARITY OF DEER AND GOAT IMMUNOGLOBULIN G (IgG)

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Abstract

A study was conducted to find out the antigenic similarity of immunoglobulin G (IgG) of sambar deer and chital to that of goats, employing enzyme linked immunosorbant assay (ELISA). Various dilutions of goat, sambar deer, chital and guinea pig sera were reacted against various dilutions of antigoat (AG) horse radish peroxidase (HRP) conjugate and the optical density values (OD) measured. Results of the study indicated that there was cross reactivity between deer and goat immunoglobulins which was evidenced by colour development in ELISA. It was also observed that the AG HRP conjugate detected chital IgG to a greater extent than sambar deer IgG. However, statistically there was significant difference between the optical OD values for the four species. It is concluded that there exists antigenic similarity between deer and goat IgG and that deer IgGs differ among themselves in their capacity to bind with AG HRP conjugate.

Key words : *Immunoglobulin G, sambar deer, chital, goat, ELISA*

Deer (Family *Cervidae*) and goats (Family *Bovidae*) are classified under Suborder *Ruminantia* of Order *Artiodactyla*. These families contain common anatomical characters like fused naviculars and cuboids, missing upper incisors to name few (Myers, 2001). Cross-reactivity of immunoglobulin molecules of different species have been used as a criterion for finding out the phylogenetic relatedness between species (Nollens *et al.*, 2008). A study was conducted to find out the antigenic similarity of IgG of sambar deer (*Rusa unicolor*) and chital (*Axis axis*) with that of goats (*Capra hircus*) employing ELISA and is hereby reported.

Materials and Methods

Sambar deer, chital, goat and guinea pig sera used in the study were obtained from the collection maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Pookode. The serum of all the four species were tested separately against antigoat (AG) IgG horse radish peroxidase (HRP) conjugates using ELISA. Goat serum was used as the homologous serum and guinea pig serum as the heterologous one (which being very much different phylogentically from goats binds minimally).

The test was carried out as per the procedure described below. Two fold dilutions of goat serum starting from (1:1000 to 1:1,28,000) were made in carbonate – bicarbonate coating buffer (pH 9.6). Hundred microlitres each of 1:1000 diluted serum was dispensed in first five wells of the first column of an ELISA plate. Similarly the next dilution (1:2000) was dispensed in first five wells of

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the second column and so on till the last dilution (column eight). The plate was incubated overnight at room temperature in a humid chamber. After the incubation, the contents of the wells were discarded and the wells washed five times with phosphate buffered saline (PBS) pH 7.4 containing 0.05% Tween 20 (PBST) and tapped dry in lint free towel. The unbound sites in each well were blocked with 200µl of 1% bovine serum albumin (BSA) in PBS (PBS BSA) for 2 h at 37°C and the plate washed and dried as before. Two fold dilutions of the AG HRP conjugate (starting from 1:1000 to 16,000) were prepared in PBST BSA (1% BSA in PBST) and 100µl each of 1:1000 dilution was loaded in the first 8 wells of the first row of the plate; 1:2000 dilution added in a similar manner to the second row and so on till the fifth row (1:16,000). The plate was incubated at 37°C for 1 h, after the incubation, washed and dried as before. Hundred microlitres of 0.04% ortho phenylenediamine dihydrochloride (OPD) in citrate buffer pH 4.0 containing 0.05% of 30 volume H_2O_2 was dispensed in all the wells in which the above reagents were added and the plate incubated for 10 min in dark at 37°C for colour development. Then 100µl each of 1.25M H_2SO_4 was dispensed into all the wells to stop color development. The optical densities (OD) of each of the wells were read using an ELISA reader at 490 nm. In a similar manner, the experiment was done separately for sambar deer, spotted deer and guinea pig sera.

The OD values for the serum dilutions (1:1000 to 1:28,000) of the four species for each dilution of AG conjugate (1: 1000, 1:2000 and so on) were analysed using single factor ANOVA to find out statistical significance (Zar, 2003).

Results and Discussion

The highest OD values for any dilution of serum/conjugate combination tested were obtained for homologous serum and conjugate *i.e.* between goat serum and AG conjugate. As expected, guinea pig serum showed very little colour development even at very low dilutions and hence negligible cross reactivity to the AG conjugate, indicating that it's IgG was very much dissimilar to the goat IgG in binding affinities. Spotted deer and sambar deer sera showed varying degrees of cross reactivity to the conjugates evidenced by varying colour development. Interestingly it was noticed that the AG conjugate distinguished sambar deer and chital serum at different levels. Spotted deer serum showed higher OD values than sambar deer serum for corresponding dilutions, indicating a greater cross reactivity with AG conjugate. Statistical analysis showed that there was significant difference ($P<0.05$) among OD values for the serum dilutions of the four species for a particular dilution of AG conjugate (Table).

Many channels of research exploring the relationship between *Ruminantia* and *Cervidae* have been undertaken. These include studies employing inhibition of radioimmunoprecipitation of immunoglobulin antigens (Curtain and Fudenberg, 1973) and the sequence analysis of mitochondrial control region (Allard *et al.*, 1992). In this study it is seen by ELISA that there was cross-reactivity between IgG of two deer species with AG conjugate indicating antigenic similarity between the IgG of these three species. Also the AG conjugate detected the IgG molecules of the two deer species studies at different extents, indicating differences in antigenic make up among the deer IgGs.

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Table. Mean OD values for sera dilutions (1:1000 to 1:1,28,000) for various dilutions of conjugate and P values for comparison by single factor ANOVA

AG conjugate dilutions	1: 1000	1:2000	1:4000	1:8000	1:16000
Goat	2.5855	2.0472	1.6131	1.1000	0.7240
Sambar	1.1205	0.60875	0.3872	0.2618	0.2103
Spotted	1.5816	0.9216	0.4963	0.2612	0.1555
Guinea Pig	0.1090	0.0866	0.0832	0.0792	0.0787
P value	1.38×10^{-10}	8.7×10^{-11}	3.1×10^{-11}	1.18×10^{-12}	4.67×10^{-13}

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