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Identification of guinea fowl red cell membrane specificities with natural haemagglutinins

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ABSTRACT

Haemagglutination with 28 phytohaemagglutinins revealed differential agglutination patterns for 5 lectins. Inheritance investigations failed to suggest genetic control. Japanese encephalitis virus antigen revealed significant age related variations in red cell agglutinability. Highest values for agglutination were observed at 1 to 15 days of age when all individuals showed high positive interaction but the incidence of non-reacting individuals increased with age. Adult phenotypes were consistent at all subsequent testings. Preliminary genetic studies with parent and progeny family groups indicated discrete genetic control.

Natural haemagglutinins have attained distinct importance due to their wide range of applicability to the problems in immunobiology and clinical pathology. Lectins, viral and tissue haemagglutinins have been used for the identification of gene determined red cell agglutinogens of farm bred avian species (Fugio and Mizutani, 1975; Mizutani *et al.*, 1977) but very little information is available regarding guinea fowl red cell agglutinogens. Shiraishi *et al.* (1979a, b) described the existence of gene determined Gf_I and Gf_{II} erythrocytic antigens using normal chicken sera agglutinins. Present investigation is an attempt to study intraspecific haemagglutination variations when tested with an array of natural haemagglutinins. Preliminary observations on inheritance of these specificities in guinea fowl are also presented.

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MATERIALS AND METHODS

Guinea fowl blood samples were obtained from mature, pedigreed 'Guncari' birds of three varieties (Lavender, Pearl and White) maintained at this institute.

Lectin extractions : Different plant seeds (2 g each) and lichen materials soaked in PBS (pH 7.2) at 4°C for 3 hr were ground to a fine suspension (1 : 10 w/w) in PBS and kept overnight at 4°C. After centrifugation at 4000 rpm for 45 min a few drops of 0.1% sodium azide was added to the filterate before storing at -20°C. Lichen extracts were also obtained from the Department of Anthropology, Punjab University, Chandigarh.

Agglutination test : The haemagglutination reaction with saline washed red cells was carried out at room temperature and the reaction was noted after 15-30 min. The quantification of agglutination reactions and inhibition of lectin were done as per Bhalla *et al.* (1978).

Testing with arbovirus : Japanese encephalitis virus (JEV) antigen was procured from National Institute of Virology, Pune. For haemagglutination test, JEV-antigen titre from 1:320 to 1:640 was used as per the method described by Clark and Casal (1958).

RESULTS AND DISCUSSION

The specific and non-specific agglutination reactions of 28 lectins (17 seeds and 11 lichens) are presented in Table 1. Strong haemagglutination tendency (100%) was apparent for most of the seed lectins, lichen extracts and fish ovary extracts. However, a considerable intraspecific agglutination variations were observed with the lectins from *Anacarodium occidentale*, *Cassia javanica*, *Cupressus macropo*, *Cupressus semeperuirens and Livistona chinensia*. Arbovirus JEV revealed discrete individual variability in agglutination (Table 2).

Repeatability studies with different blood collections from the same individuals revealed no differences but enhanced reactivity was evident with blood cell samples tested after storage. A loss of agglutinating activity of lichen extracts, however, was observed during storage. This might lead to discrepancies and reduced precision in actual practice. Possibly, such activity losses occur because lectins are inherently unstable. Alternatively, it may be destroyed by proteases that often contaminate the lichen substrate (Judd, 1989). A similar loss of agglutinating activity has also been reported by Andrews *et al.* (1992).

The agglutinability of chicken red cells was also found to be influenced by estrogen status (Salmeinen, 1962; Scheinberg and Reckel, 1962) but no such dimorphism was identified in the present survey. Varietal differences in the incidence of agglutinogens detected by lectins were not statistically significant Agglutination reaction of *Glycin max* seed extracts was very strong with chicken and quail RBCs but only modrate in case of guinea fowl red cells. Existence of similar species-specific differences has been reported by Scheinberg and Reckel (1962) and McPhee (1970).

Discrete Japanese encephalitis virus antigen erythrocyte interaction was detected in majority of guinea fowl samples; about 21% of the individuals did not show agglutination reaction (Table 2). The individual variations were also observed in cell-virus reactivity. Different individuals showed different titre values. Age related variations were also identified; the RBCs of young keets (0-15 days) showed high reactivity with the virus as compared to the adults (Fig. 1). Repeated agglutination pattern assay of six high titre group growers revealed stable values. The observed differences in agglutination reactions were all statistically non-significant.

Preliminary observations regarding inheritance of the agglutination reactions of guinea fowl red cells with Japanese encephalitis virus (JEV) showed single gene control, possibility for this trait (Table 3). Using vaccinia virus Gilmour (1959) identified a chicken red cell agglutinogen 'V'h'; its inheritance

Table 1. Haemagglutination reactions of guinea fowl red cells with lectins

Lectin source	Agglutination reaction type	Percentage positive
Plant seeds :		
Anacarodium occidentale	++	20
Anthocephalus uiticus	–	0
Banhinia purpurea	–	0
Bischofia javanica	–	0
Cassia javanica	+	11
Cassia spectabilitis	++	100
Cupressus macropo	++	48
Cupressus semeperuirens	±	19
Leucaena leucocephala	+	100
Lowsonia alba	++	100
Livistona chinensia	+	60
Phoenix rupicola	+	98
Pterospermum acerifolicum	–	0
Ricinus communis	+++	100
Robinia psedoacacia	+++	100
Pisum sativum	+++	100
Glycin max	+	100
Cactus stems :		
Euphrobia triradiate	+	100
Mushrooms :		
Aganicus basianulosum	+++	100
Cantharellus cibarius	+	100
Geastrum assenarinus	+	100
Morchella esculenta	+++	100
Pleurotus citrinopiteatus	+	100
Polyporus singuinis	+	100
Pleunotus eousmus	+	100
Russnla alutacea	+	100
Volvaria diplasia	+	100
Algae :		
Zygonanium indicum	+	100
Fish ovary :		
Labeo rohita	+	100
Cyprinus carpio	+++	100

Table 2. Guinea fowl red cell agglutination profile with JEV antigen (N-117)

Titre value	Individuals tested	Percentage observed
- Ve	25	21.37
1 : 40	15	12.82
1 : 80	22	18.80
1 : 160	40	34.19
1 : 320	10	8.55
1 : 640	5	4.27

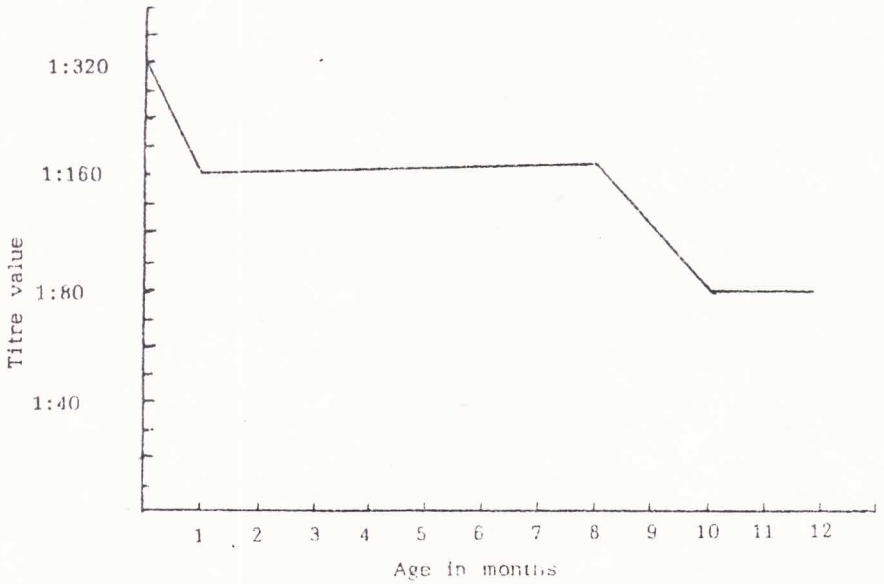

Fig. 1. Age related variations in red cell agglutination with JEV antigen.

Table 3. JEV haemagglutination titre observed among offsprings from matings between parents showing high, medium and low titre values

Parents				Offsprings		
Sire		Dam	N	Low (1:0-40)	Medium (1:40-80)	High (1:80 and above)
-Ve (4)	x	-Ve (4)	13	12	1	—
-Ve (3)	x	High (3)	12	2	2	8
High (3)	x	High (4)	15	—	2	13

(In parenthesis is the number of sires/dam used).

studies revealed dominance of presence of the receptor (Vh) over its absence. Breed studies by Petrovsky (1977) revealed wide diversity in the incidence of 'Vh' agglutinogen, and relatively higher frequency of Vh allele was observed among the layer populations as compared to meat strains. In another study, McPhee (1970) described the existence of an autosomal dominant gene in the presence of which chicken red cells were agglutinated by Kahan's antigen. Its influence on fertility and hatchability was also reported. However, no such trends were detected among the three guinea fowl genetic groups. These inferences need further substantiation with larger data in view of limited pedigreed population size included in this investigation.

Lectins are soluble proteins or glycoproteins which interact in a variety of ways with red cell membrane receptors, by virtue of their specific binding affinities to certain carbohydrate moities. Not enough attention has been placed on the search for blood group-specific lectins for alternate poultry species, although lectins with apparent or confirmed blood group specificity have been reported for chicken, cattle, goats, dogs, cats and fish (Judd, 1989; Andrews *et al.*, 1992). In view of the difficulties involved in production of specific isoimmune blood grouping reagents and their doubtful reproducibility when obtained from individuals of heterogenous genetic stocks, more extensive investigations on these lines are highly desired.

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