

Further Evidence of Linkage at the *tva* and *tvc* Loci in the Layer Lines and a Possibility of Polyallelism at the *tvc* Locus

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ABSTRACT : Three lines of White Leghorn (WL) chickens (IWJ, IWG and IWC) maintained at Central Avian Research Institute, Izatnagar (UP), were used for chorioallantoic membrane (CAM) and liver tumour (LT) assay. Eleven-day-old embryos of each line were partitioned into three groups and inoculated with 0.2 ml of subgroup A, subgroup C and an equal mixture of subgroup A and C Rous sarcoma virus (RSV). Subgroup virus receptor on the cell surface membrane for subgroup A is coded for by tumour virus a (*tva*) locus and for subgroup C by tumour virus c (*tvc*) locus. The random association of the genes at the *tva* and *tvc* loci in IWJ and IWC line was assessed and the χ^2 -values for phenotypic classes were found to be significant, indicating the linkage between the *tva* and *tvc* loci. The linkage value was estimated to be 0.09 on pooled sex and pooled line basis. On the basis of four subclass tumour phenotypes a 4-allele model was proposed for *tva* locus having a^{s1} , a^{s2} , a^{r1} and a^{r2} alleles and the frequencies were calculated as 0.47, 0.13, 0.13 and 0.27 for IWJ line, 0.31, 0.33, 0.14 and 0.22 for IWG line and 0.44, 0.11, 0.21 and 0.24 for IWC line, respectively. Similarly, for *tvc* locus the frequencies of four alleles i.e. c^{s1} , c^{s2} , c^{r1} and c^{r2} were calculated as 0.42, 0.20, 0.21 and 0.17 for IWJ line, 0.42, 0.17, 0.27 and 0.14 for IWG line and 0.30, 0.21, 0.16 and 0.33 for IWC line, respectively. The χ^2 -values for all classes of observations were not significant ($p > 0.05$), indicating a good fit to the 4-allele model for the occurrence of 4-subclass tumour phenotypes for *tva* and *tvc* loci. On the basis of the 2-allele model both *tva* and *tvc* locus carries three genotypes each. But, on the basis of the 4-allele model *tva* and *tvc* locus carries 10 genotypes each. The interaction between A-resistance and C-resistance (both CAM and LT death) was ascertained by taking the 10 genotypes of *tva* locus and 3 genotypes of *tvc* locus by pooling the lines and partitioning the observations into 3 classes. The χ^2 -values for the genotypic classes of CAM (-) LT (+) and CAM (-) LT (-) phenotypes to mixed virus (A+C) infection were found to be highly significant ($p < 0.01$), indicating increased resistance, which indicates the joint segregation of a' and c' genes, suggesting the existence of close linkage between the *tva* and *tvc* loci. Therefore, an indirect selection approach using subgroup C viruses can be employed to generate stocks resistant to subgroup A LLV, obviating contamination with the most common agent causing LL in field condition. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 5 : 601-605)

Key Words : Chorioallantoic Membrane, Liver Tumour, Linkage, Polyallelism, Tumour Virus

INTRODUCTION

Lymphoid Leukosis (LL) in chickens is caused by avian leukosis virus (ALV). The prevalence of the disease is world wide and still a challenging problem to modern poultry enterprises due to the lack of usual disease control measures such as vaccination and therapeutics. LL diseases that occur in field condition is mostly caused by lymphoid leukosis virus subgroup A (LLV-A). One possible approach to eradicate LLV-A from chicken flocks is to develop resistant stock of the $a'a'$ genotype that does not permit virus infection (Payne, 1985). Under laboratory condition the study of genetic control mechanism of LL disease caused by LLV-A is very complex because of difficulties in controlling environmental variations due to a longer incubation period. On the other hand, Rous sarcoma virus of subgroup A (RSV-A) that is related to LLV-A

antigenically, for host range and interference pattern (Pani, 1976) induces liver tumour resulting in early death in chicks that are infected via the chorioallantoic membrane (CAM) on the 11th day of incubation (Pani et al., 1988; Pani and Naithani, 1990). Hence, the studies of genetic control mechanism for leukosis disease in chickens, RSV-A as a model virus is usually being used for the above reasons. Pani and Naithani (1990) reported that pocks on CAMs is not an efficient index of resistance to infection by leukosarcoma viruses of subgroup A because a good proportion of chicks that developed from eggs without pocks in CAMs also died of liver tumour (LT) and also chicks developed from eggs with pocks in CAMs did not die of LT. Hence, a 4-allele model has been suggested by Pani and Naithani (1990 and 1991) to control LT phenotype in some heavy and light exotic breeds. Incidence of subgroup C viruses in field as per reports available is very rare. Therefore, subgroup C viruses can be regarded as laboratory variants.

Subgroup virus receptor on the cell surface membrane for subgroup A is coded for by tumour virus a (*tva*) locus and for C, by tumour virus c (*tvc*) locus. Host cell genes of *tva* locus have been shown to be linked with that of *tvc*

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locus (Payne and Pani, 1971; Pani, 1974; Dren and Pani, 1977). This study has, therefore, been conducted to investigate interrelationship between host cell genes of *tva* and *tvc* loci in layers selected for high egg production for over ten generations. This may enable selection for C-resistance for developing an A-resistant stock, thus obviating the contamination with a field virus (subgroup-A). This study also provides a hypothesis that the *tvc* locus could be polyallelic similar to the *tva* locus, as reported by Pani and Naithani (1991) and suggests a possible interaction between A-resistance and C-resistance in terms of polyallelism.

MATERIALS AND METHODS

Chicken embryos and virus strains

Embryos were collected from three lines of White Leghorn chicken (IWJ, IWG and IWC) maintained by artificial insemination with 1 male to 4-7 females in conventional cage houses at Central Avian Research Institute (CARI), Izatnagar (Ghosh and Pani, 2002). The IWJ and IWG lines procured in the year 1972 from Israel and USA and selected for high egg production for about ten generation using Osborne's index selection and the IWC line was developed at CARI by random breeding (Johari et al., 1992).

Bryan standard strain of Rous sarcoma virus (BS-RSV) (titre 10^6 pfu/ml) of subgroup A and pseudotype of Brayan high titre [RSV(RAV-49)] of subgroup C (titre 10^6 pfu/ml) obtained from Dr. L. N. Payne, Houghton Poultry Research Station, (UK) were used. Stock solutions were diluted to contain 10^3 pfu/ml in both the virus subgroups with primary cell culture media.

Experimental design

An approach has been made to ascertain linkage between subgroup A and C viruses by using CAM-assay. The 11-day-old embryos were divided into three groups (line wise). Subgroup A virus, subgroup C virus and an equal mixture of subgroup A and subgroup C virus had been inoculated to the three groups.

CAM- and LT-assay

Conventional CAM- and LT-assay were conducted as per the published procedure (Dougherty et al., 1960; Pani et al., 1988; Ghosh and Pani, 2002). The dead chicks that had nodules in the liver were considered as liver tumour positive [LT (+)] and those that had clean liver without visible gross tumour as liver tumour negative [LT (-)] phenotype. The chicks survived after 8 weeks were assumed as liver tumour negative [LT (-)]. Line wise record of LT (+) LT (-) and CAM (+) CAM (-) of dead chicks were maintained for analysis.

Statistical analysis

Gene frequency at the *tva* and *tvc* loci were calculated using the formula:

$$f(a') = \left[\frac{n(C/A)}{N} \right]^{1/2} \quad f(a^s) = 1 - f(a')$$

$$f(c') = \left[\frac{n(C/C)}{N} \right]^{1/2} \quad f(c^s) = 1 - f(c')$$

(Falconer and Mackay, 1989)

Where, $n(C/A)$ and $n(C/C)$ represents number of embryos resistant to subgroup A and subgroup C viruses, respectively.

n -stands for number of observations in the phenotypic class. N - the total number of observations.

Genotypes and phenotypic frequencies are represented as follows-

Genotypes	Phenotypic frequencies
(i) $a^s a^s c^s c^s$	$f(C/AC)$
(ii) $a^s a^s c^s -$	$f[(C/A)-(C/AC)]$
(iii) $a^s - c^s c^s$	$f[(C/C)-(C/AC)]$
(iv) $a^s - c^s -$	$1 - [f(C/AC) - f(C/A) - (C/AC)] + f[(C/C) - (C/AC)]$

Where, f -stands for number of embryos of subclass C/A, C/C and C/AC, observed to be resistant to single infection by subgroup A, C and mixed virus infection, respectively.

Chi-square (χ^2) test (Snedecor and Cochran, 1967) was used to test the random association of genes at *tva* and *tvc* loci, fitting of 4-allele model for the occurrence of four subclass tumour phenotypes and to ascertain interaction between C-resistance and A-resistance in terms of polyallelism.

RESULTS AND DISCUSSION

The proportions of four possible phenotypes (C/O, C/A, C/C and C/AC) with genotypes ($a^s c^s$, $a^s c^s c^s$, $a^s a^s c^s$ and $a^s a^s c^s c^s$) in respect of *tva* and *tvc* loci are presented in Table 1 for IWJ and IWC lines. The χ^2 -values for phenotypic classes were found to be either significant ($p < 0.05$) or highly significant ($p < 0.01$), indicating a linkage between *tva* and *tvc* loci.

Payne (1972) suggested that the a^s gene may be beneficial to the organism and is possibly favoured by natural selection. In that case the simultaneous increase in the frequencies of a^s and c^s genes could have been expected which indeed has occurred in this study, supporting thereby the linkage hypothesis between *tva* and *tvc* loci (Payne and Pani, 1971). Pani (1974) on examination of the F_2 embryo cultures infected with BS-RSV and RSV (RAV-49), either

Table 1. Chi-square (χ^2) test to assess the random association of the genes at the *va* and *vc* loci in IWJ and IWC lines of White Leghorn chicken

Pheno-type	Genotype	IWJ					IWC				
		Obs. ¹ Prop.	Exp. ² Prop.	Obs. number	Exp. number	χ^2	Obs. ¹ Prop.	Exp. ² Prop.	Obs. number	Exp. number	χ^2
C/O	a^s-c^s	0.82	0.72	103	90.72	1.66 ^{NS}	0.80	0.58	68	49.30	7.09**
C/A	a^s-c^s	0.06	0.15	8	18.90	6.29*	0.07	0.14	6	11.90	2.93 ^{NS}
C/C	a^s-c^s	0.03	0.11	4	13.86	7.01**	0.02	0.23	2	19.55	15.75**
C/AC	a^s-c^s	0.09	0.02	11	2.52	28.54**	0.11	0.05	9	4.25	5.31*
Total		1.00	1.00	N=126			1.00	1.00	N=85		

Obs.: Observed; Exp.: Expected; Prop.: Proportion.

¹ As per test.² The expected genotypic and phenotypic frequencies were calculated using the frequencies 0.58, 0.42, 0.64 and 0.36 for a^s , a^r , c^s and c^r genes, respectively for IWJ line and 0.56, 0.44, 0.47 and 0.53 for a^s , a^r , c^s and c^r genes, respectively for IWC line of WL-chicken.N: The geometric mean of the total number of CAMs infected with subgroup A virus (n_1) and total number of CAMs infected with subgroup C virus (n_2); i.e. $N = \sqrt{(n_1 \times n_2)}$ NS: Not significant. * $p < 0.05$, ** $p < 0.01$.**Table 2.** The segregation of susceptible and resistant phenotype within LT subclasses for IWJ, IWG and IWC lines and χ^2 analysis showing the fitness of 4-allele model to the *va* locus

Tumour phenotypes	IWJ	IWG	IWC	Total	Lines	Allelic frequency				
						a^{s1}	a^{s2}	a^{r1}	a^{r2}	Total
CAM(+)LT(+)	49	42	32	123	IWJ	0.47	0.13	0.13	0.27	1.00
CAM(+)LT(-)	2	5	2	9	IWG	0.31	0.33	0.14	0.22	1.00
CAM(-)LT(+)	1	1	2	4	IWC	0.44	0.11	0.21	0.24	1.00
CAM(-)LT(-)	9	6	7	22						
Total	61	54	43	158						
$f(a^s)$	0.40	0.36	0.45							

Tumour phenotypes	IWJ			IWG			IWC		
	Observed number	Expected number	χ^2	Observed number	Expected number	χ^2	Observed number	Expected number	χ^2
CAM(+)LT(+)	49	47.52	0.05 ^{NS}	42	42.06	0.00 ^{NS}	32	33.5	0.07 ^{NS}
CAM(+)LT(-)	2	3.48	0.63 ^{NS}	5	3.08	1.20 ^{NS}	2	2.45	0.08 ^{NS}
CAM(-)LT(+)	1	1.33	0.08 ^{NS}	1	1.35	0.09 ^{NS}	2	1.09	0.76 ^{NS}
CAM(-)LT(-)	9	8.48	0.03 ^{NS}	6	7.51	0.30 ^{NS}	7	5.99	0.17 ^{NS}
Total	61			54			43		

NS: Not significant; CAM: Chorioallantoic membrane; LT: Liver tumour.

alone or mixed, found that the segregation of a^s and a^r genes and that of c^s and c^r genes, did not differ significantly ($p > 0.05$) from the expected 3:1 ratio in χ^2 -test of heterogeneity. But when the allelic pair of *va* and *vc* loci were considered at a time for 15:1 segregation ratio, the χ^2 -test of heterogeneity showed a highly significant value ($p < 0.01$), indicating a linkage between *va* and *vc* loci.

In our present study, the observed and expected proportions were found to be significantly different ($p < 0.01$) for the four possible phenotypes i.e. C/O, C/A, C/C and C/AC, in IWJ and IWC lines of WL chicken, indicating the absence of random association between the genes at the two loci. This can be taken as an evidence for close association between the two tumour virus loci, *va* and *vc*. The observed proportions of C/AC phenotypes of the three lines were very near to the products of the frequency of a^s gene i.e. $[f(a^s)]^2$ or c^s gene i.e. $[f(c^s)]^2$, instead of $[f(a^s) \cdot f(c^s)]^2$, suggesting strongly either the pleiotropic control of the phenotype by a single locus or a very close linkage between

the two loci. The pleiotropic control of the C/AC phenotype has been ruled out by previous workers (Pani, 1974; Dren and Pani, 1977) and hence, it can be taken as an evidence for the close linkage between two loci. The linkage value was estimated to be 0.09 on pooled sex and pooled line basis (Table 1), which agreed well with the linkage value of 0.08 ± 0.03 , on a pooled sex basis, as reported by Pani (1974) in light breed of chicken.

Subgroup A virus infection resulted in four possible subclasses of tumour phenotypes as reported by Pani et al. (1988). On the basis of four subclass tumour phenotypes, a 4-allele model according to Pani and Naithani (1990), for *va* locus was fitted in this present study. According to the 4-allele model, *va* locus carries four alleles, two susceptibility allele (a^{s1} and a^{s2}) and two resistance alleles (a^{r1} and a^{r2}). The frequencies of a^{s1} , a^{s2} , a^{r1} and a^{r2} alleles were calculated as 0.47, 0.13, 0.13 and 0.27 for IWJ line, 0.31, 0.33, 0.14 and 0.22 for IWG line and 0.44, 0.11, 0.21 and 0.24 for IWC lines, respectively (Table 2). The χ^2 -

Table 3. The segregation of susceptible and resistant phenotype within LT subclasses for IWJ, IWG and IWC lines and χ^2 analysis showing the fitness of 4-allele model to the *trc* locus

Tumour phenotypes	IWJ	IWG	IWC	Total	Lines	Allelic frequency				Total
						c^{s1}	c^{s2}	c^{r1}	c^{r2}	
CAM (+) LT (+)	64	47	52	163	IWJ	0.42	0.20	0.21	0.17	1.00
CAM (+) LT (-)	7	6	5	18	IWG	0.42	0.17	0.27	0.14	1.00
CAM (-) LT (+)	4	5	2	11	IWC	0.30	0.21	0.16	0.33	1.00
CAM (-) LT (-)	8	6	16	30						
Total	83	64	75	222						
f(c^r)	0.38	0.41	0.49							

Tumour phenotypes	IWJ			IWG			IWC		
	Observed number	Expected number	χ^2	Observed number	Expected number	χ^2	Observed number	Expected number	χ^2
CAM (+) LT (+)	64	60.94	0.15 ^{NS}	47	46.90	0.00 ^{NS}	52	55.06	0.17 ^{NS}
CAM (+) LT (-)	7	6.73	0.01 ^{NS}	6	5.19	0.13 ^{NS}	5	6.08	0.19 ^{NS}
CAM (-) LT (+)	4	4.11	0.00 ^{NS}	5	3.17	1.06 ^{NS}	2	3.72	0.79 ^{NS}
CAM (-) LT (-)	8	11.22	0.92 ^{NS}	6	8.65	0.81 ^{NS}	16	10.14	3.38 ^{NS}
Total	83			64			75		

NS: not significant; CAM: Chorioallantoic membrane; LT: Liver tumour.

Table 4. Interaction between C-resistance and A-resistance in terms of polyallelism in White Leghorn chicken (Pooled line basis)

Genotypes	Phenotypes	Observed number	Expected number	χ^2
$a^{s1} - c^s -$ $a^{s2} - c^s -$	C/O	45	54.68	1.71 ^{NS}
$a^{r1} a^{r1} c^s -$ $a^{r1} a^{r2} c^s -$ $a^{r2} a^{r2} c^s -$				
$a^{s1} - c^r c^r$ $a^{s2} - c^r c^r$	C/C	3	0.28	26.42**
$a^{r1} a^{r1} c^r c^r$ $a^{r2} a^{r2} c^r c^r$				
$a^{r1} a^{r2} c^r c^r$ $a^{r2} a^{r2} c^r c^r$	C/A''C	8	1.04	46.58**
Total		56	56	

NS: Not significant; ** $p < 0.01$.

C/A''C=CAM (-) LT (-) phenotypes in mixed virus (A-C) infection.

C/A''C=CAM (-) LT (-) phenotypes in mixed virus (A-C) infection.

The expected proportions were calculated using frequencies 0.41, 0.19, 0.16 and 0.24 for a^{s1} , a^{s2} , a^{r1} and a^{r2} genes, respectively and 0.56 and 0.44 for c^s and c^r genes, respectively.

values in all classes of observations were not significant ($p > 0.05$), indicating a good fit to the 4-allele model of the *trc* locus.

Similarly, the occurrence of 4-subclass tumour phenotypes i.e. CAM (+) LT (+), CAM (+) LT (-), CAM (-) LT (+) and CAM (-) LT (-) infected with subgroup C virus was also observed and a 4-allele model was proposed for the *trc* locus. On the assumption of a 4-allele model similar to the *trc* locus, the frequencies of c^{s1} , c^{s2} , c^{r1} and c^{r2} alleles of the *trc* locus were calculated as 0.42, 0.20, 0.21 and 0.17 for IWJ line, 0.42, 0.17, 0.27 and 0.14 for IWG line and 0.30, 0.21, 0.16 and 0.33 for IWC line, respectively (Table 3). In this case the χ^2 -values in all classes of observations were also not significant ($p > 0.05$), indicating a good fit to

the 4-allele model of the *trc* locus. The occurrence of 4 subclass tumour phenotypes of subgroup C in the three lines (IWJ, IWG and IWC) with non-significant ($p > 0.05$) χ^2 -value indicated the role of 4-allele model to the *trc* locus also similar to the proposed 4-allele model (Pani and Naithani, 1990) for genetic control of CAM infection in embryos and LT death in chicks to subgroup A virus. Furthermore, the 4-allele model of the *trc* locus in the present study also provided support to the earlier report of Pani and Naithani (1990).

On the basis of 2-allele model, the *trc* locus carries a^s and a^r alleles and 3 genotypes ($a^s a^s$, $a^s a^r$ and $a^r a^r$) and similarly, the *trc* locus also carries c^s and c^r alleles and 3 genotypes ($c^s c^s$, $c^s c^r$ and $c^r c^r$). Based on proposed 4-allele model, the *trc* locus carries at least 4 alleles (a^{s1} , a^{s2} , a^{r1} and a^{r2}) and 10 genotypes ($a^{s1} a^{s1}$, $a^{s1} a^{s2}$, $a^{s1} a^{r1}$, $a^{s1} a^{r2}$, $a^{s2} a^{s2}$, $a^{s2} a^{r1}$, $a^{s2} a^{r2}$, $a^{r1} a^{r1}$, $a^{r1} a^{r2}$ and $a^{r2} a^{r2}$) and similarly, the *trc* locus also carries at least 4 alleles (c^{s1} , c^{s2} , c^{r1} and c^{r2}) and 10 genotypes ($c^{s1} c^{s1}$, $c^{s1} c^{s2}$, $c^{s1} c^{r1}$, $c^{s1} c^{r2}$, $c^{s2} c^{s2}$, $c^{s2} c^{r1}$, $c^{s2} c^{r2}$, $c^{r1} c^{r1}$, $c^{r1} c^{r2}$ and $c^{r2} c^{r2}$). To study the linkage between *trc* and *trc* loci, the interaction between C-resistance and A-resistance (both CAM and LT death) was ascertained by taking the 10 genotypes of *trc* locus and 3 genotypes of *trc* locus by pooling the lines and by partitioning the observations in to three classes (Table 4). As the occurrence of phenotypic observations was of uniform frequency in each class of the three lines, the data of the three lines were pooled. The χ^2 -value for the genotypic classes of CAM (-) LT (+) phenotypes and CAM (-) LT (-) phenotypes to mixed virus (A+C) infection was found to be highly significant ($p < 0.01$) (Table 4). This indicates the unexpected occurrence of phenotypes with lack of Mendelian pattern of segregation. The addition of subgroup A to subgroup C virus increased the resistance than infection by subgroup A virus alone. The increased resistance in mixed virus

infection indicated the joint segregation of *a'* and *c'* genes. This type of phenomenon suggested that there might exist close linkage between *tva* and *tvb* loci. This gives an additional support of linkage between *tva* and *tvb* loci which was reported earlier on the basis of CAM phenotypes (R and S) in 2-allele model of *tva* and *tvb* loci (Pani, 1974). Therefore, an indirect selection approach using subgroup C viruses can be employed to generate stocks resistant to subgroup A LLV, obviating contamination with the most common agent causing LL in field condition.

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