

## SUMMARY

The chicken's major histocompatibility complex (MHC) haplotype has profound influence on the resistance or susceptibility to certain pathogens such as B21 MHC haplotype confers resistance to Marek's disease (MD). However, non-MHC genes are also important in disease resistance. For example, both lines 6 and 7 both express the B2 MHC haplotype but differ in non-MHC genes. Line 6, but not line 7, is highly resistant to tumors induced by the Marek's disease herpesviruses and avian leukosis retroviruses. Recently, survival in the field by Thai indigenous chickens to H5N1 high pathogenicity avian influenza (HPAI) outbreaks was attributed to B21 MHC haplotype while the B13 MHC haplotype was associated with high mortality in the field. To determine the influence of the MHC haplotype on HPAI resistance, a series of MHC congenic white leghorn chicken lines (B2, B12, B13, B19 and B21) and lines with different background genes but with the same B2 MHC haplotype (Line 63 and 71) were intranasally challenged with low dose (10 mean chicken lethal doses) of H5N1 HPAI virus rgA/chicken/Indonesia/7/2003. None of the lines were completely resistant to lethal effects of the challenge as evident by mortality rates ranging from 40 to 100%. The B21 line had mortality of 40% and 70% and the B13 line had mortality of 60 and 100% in 2 separate trials. In addition, the mean death times varied greatly between groups, ranging from 3.7 to 6.9 days suggesting differences in pathogenesis. The data show that the MHC has some influence on the resistance to AI, but less than previously proposed, and non-MHC background genes may have a bigger influence on resistance than the MHC.

## INTRODUCTION

A retrospective survey of the MHC haplotypes obtained from live and dead indigenous chickens in regions of Thailand known to have outbreaks of H5N1 HPAI reported that 100% of the B21 haplotype was present in survivors and none in any of the fatal cases.

LHK Line			PHD Line		
Haplotypes	No. chickens	% Survival	Haplotypes	No. chickens	% Survival
A1A1	10	0.00	A1A1	18	0.00
B12B12	33	0.00	B12B12	111	2.70
B12B13	21	4.76	B12B13	14	7.14
<b>B12B21</b>	<b>18</b>	<b>100.00</b>	<b>B12B21</b>	<b>8</b>	<b>100</b>
B13B13	44	0.00	B13B13	50	0.00
B13B15	19	0.00	B13B15	27	0.00
B13B2	8	0.00	B13B2	10	0.00
B13BA12	4	0.00	B13BA12	4	0.00
B15B2	9	77.77	B15B2	7	100.00
<b>B15B21</b>	<b>26</b>	<b>100.00</b>	<b>B15B21</b>	<b>10</b>	<b>100.00</b>
B15B5	10	30.00	B15B5	6	33.33
<b>B19B19</b>	<b>9</b>	<b>22.22</b>	<b>B19B19</b>	<b>5</b>	<b>80.00</b>
B19B5	10	50.00	B19B5	8	50.00
<b>B21B21</b>	<b>53</b>	<b>100.00</b>	<b>B21B21</b>	<b>121</b>	<b>100.00</b>
B21BA12	16	87.50	B21BA12	16	87.50
<b>B2B21</b>	<b>9</b>	<b>100.00</b>	<b>B2B21</b>	<b>7</b>	<b>100.00</b>

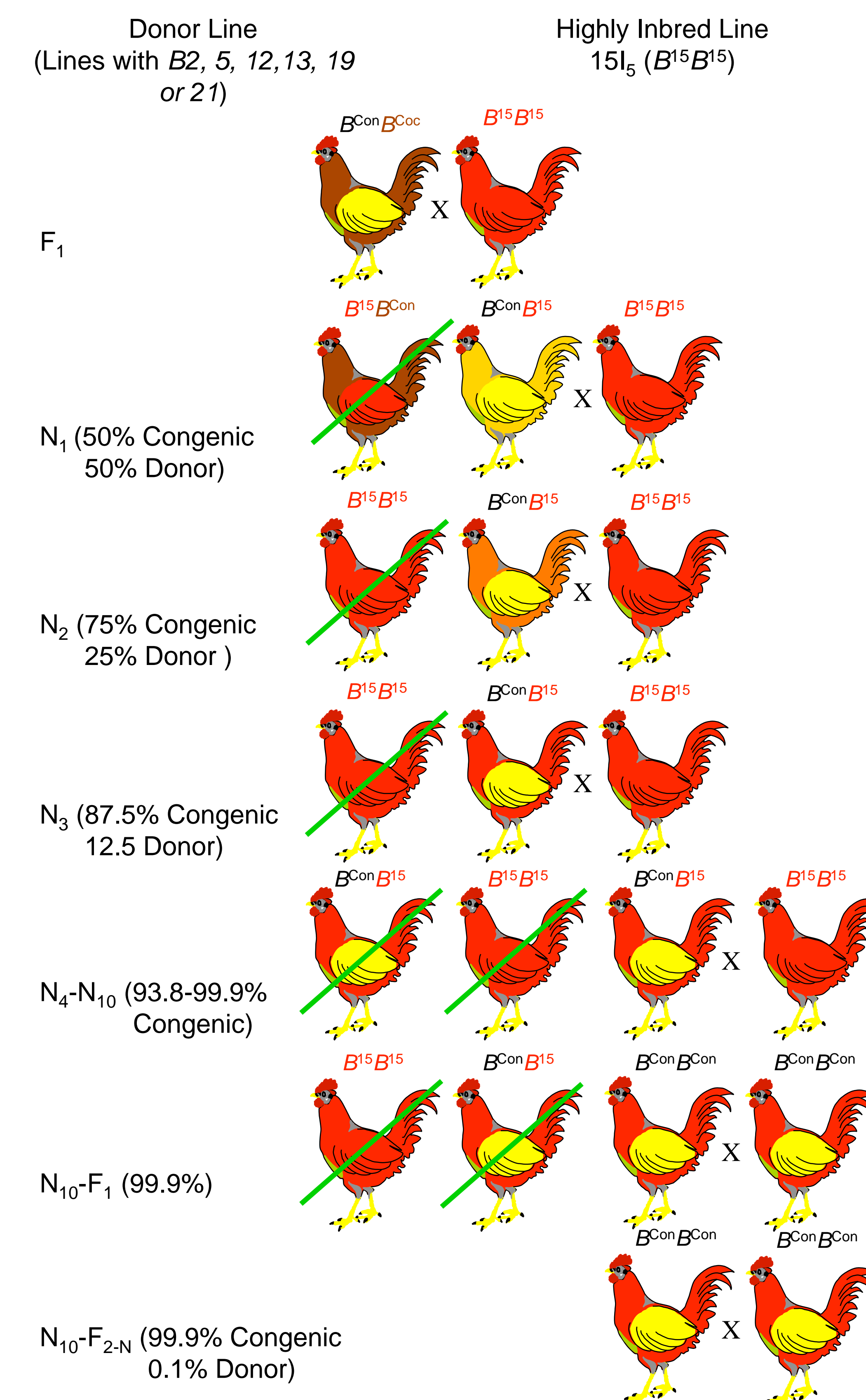
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The authors conclude that the MHC B21 haplotype is highly resistant to HPAI strain H5N1. Although the infection status of individual chickens was not determined, the results suggested that the MHC haplotype may be linked to resistance or susceptibility to H5N1. To test the influence of the B haplotype on resistance to HPAI we challenged two different generations of B congenics with H5N1 HPAI strain

## MATERIALS AND METHODS

The 15-B congenic lines were developed by 10 or 11 backcross generations of mating to inbred line 15I<sub>5</sub>. Male breeders heterozygous for an introduced B haplotype were selected for 10 to 11 backcrosses to 15I<sub>5</sub>. B-heterozygous parents were mated and chickens homozygous for the introduced gene were selected. Since development, 6 males and 30 to 50 females have been used to reproduce each line for 19 generations. Before each backcross the males and females of each line are typed to insure the correct B haplotype.

Each of the 15.B congenic lines are greater than 99.9% identical to the inbred parental 15I<sub>5</sub> chicken line, but each is homozygous for the unique set of genes defining the individual B-haplotypes



To characterize the effects of different B-complex haplotypes on HPAI resistance, four 15-B congenic lines, 15.C-12 (B12), 15.P-13 (B13), 15.P-19 (B19), 15.N-21 (B21) and the parental 15I<sub>5</sub> (B15) lines were used in two separate trials. In addition, we included three lines with the same B haplotype but quite different background genes. To compare the effects of different background genes on the resistance or susceptibility of HPAI we included the B2 haplotype the B congenic line 15.7-2 (B2), inbred line 7<sub>1</sub> (B2) and inbred line 6<sub>3</sub> (B2).

To determine the influence of the B complex on HPAI, the reverse genetic derived strain rgA/chicken/Indonesia/7/2005 (H5N1) HPAI virus was used as the challenge virus and administered at a minimal chicken lethal dose that would produce 100% mortality; i.e. 10 mean chicken lethal doses (CLD<sub>50</sub>). Based on preliminary experiments, 10 CLD<sub>50</sub> was equivalent to 10<sup>3</sup> mean embryo infectious doses (EID<sub>50</sub>). In the two trials, based on back titrations, each 3 week old chicken received 10<sup>3.1</sup> (trial 2) or 10<sup>3.3</sup> (trial 1) EID<sub>50</sub> of virus in 0.1 ml via inoculation through the choanal slit into the middle nasal chamber.

## RESULTS AND DISCUSSION

The MHC haplotypes B2, 5, 12, 13 19 and 21 were challenged and monitored for disease and mortality induced by the H5N1 challenge. The results of trials 1 and 2 (see below) give the percent mortality, the number of birds alive per days post infection and the mean time to death for the different B congenic haplotypes.

In the first trial, mortality ranged from 50% (B5) to 100% (B13) with 70% mortality observed in the B21 haplotype. In the second trial, the mortality rates ranged from 40% (B19 & B21) to 80% (B5) in the congenic groups. The mortality rate for B5 group was significantly shorter than for B13 group in trial one, but mortality rates between other groups were not statistically different in trial one and mortality rates were not different between any groups in trial 2

Trial 1.

Genetic Line (B Haplotype)	Number Challenged	Mortality (10 DPI) % Mort	Number Alive at DPI										Rank (MDT <sup>2</sup> (MDT>))
			3	4	5	6	7	8	9	10	MDT		
15.P-13 (B13)	10	10 <sup>ab</sup>	100	5	2	2	1	1	0	0	0	4.1	4
15.C-12 (B12)	10	6 <sup>ab</sup>	60	8	4	4	4	4	4	4	4	3.7	1
15.15I5 (B15)	10	5 <sup>b</sup>	50	9	6	5	5	5	5	5	5	4.0	3
15.P-19 (B19)	10	9 <sup>ab</sup>	90	7	2	2	2	2	1	1	1	4.1	4
15.N-21 (B21)	10	7 <sup>ab</sup>	70	8	4	3	3	3	3	3	3	3.9	2

Trial 2.

Genetic Line (B Haplotype)	Number Challenged	Mortality (10 DPI) % Mort	Number Alive at DPI										Rank (MDT <sup>2</sup> (MDT>))
			3	4	5	6	7	8	9	10	MDT		
15.P-13 (B13)	10	6 <sup>a</sup>	60	10	8	7	6	4	4	4	4	5.5	4
15.C-12 (B12)	10	6 <sup>a</sup>	60	9	7	7	6	5	5	5	5	4.8	2
15.15I5 (B15)	10	8 <sup>a</sup>	80	9	5	4	3	3	2	2	2	4.8	2
15.P-19 (B19)	10	4 <sup>a</sup>	40	10	8	7	7	7	6	6	6	5.5	3
15.N-21 (B21)	10	4 <sup>a</sup>	40	9	6	6	6	6	6	6	6	3.8	1
15.7-2 (B2)	10	4 <sup>a</sup>	40	9	7	6	6	6	6	6	5	4.0	
6 <sub>3</sub> (B2)	10	7 <sup>a</sup>	70	9	8	7	7	5	5	5	5	5.2	
7 <sub>1</sub> (B2)	10	9 <sup>a</sup>	90	10	9	7	7	4	3	2	1	6.9	

<sup>1</sup>Days Post Infection

<sup>2</sup>Mean Death Time

<sup>3</sup> Statistical tests – different lower superscript letters denote statistical significance, Fisher Exact test, P<0.05.

When examining the mean survival times (MST) for birds in each group, the B13 haplotype had statistically significant shorter survival times than the B5 or B12 haplotypes but this significance did not repeat in the second trial (Table 2). The variability between the two trials does not allow the ranking of the different B haplotypes with regard to resistance or susceptibility, note that the most resistant B haplotype (B5) in the first trial was the most susceptible in the second trial. The survival times between the B5 and B19 haplotypes was statistically different in both trials (Table 2) however, as noted above, in trial 1 the B5 was the most resistant whereas in trial two B5 was the most susceptible. The results do show that all B haplotypes tested in this study, including the B21 haplotype, are susceptible to death induced by H5N1 virus challenge. In the second trial (Table 1), we investigated the effects of background genes on resistance or susceptibility to H5N1. Chicken lines 15.7-2, 6<sub>3</sub> and 7<sub>1</sub> have the B2 MHC haplotype but vary significantly in their background genes. Within the different B2 haplotypes, mortality ranged from 40% in the 15.7-2 line to 90% in line 7<sub>1</sub> with 70% mortality in line 6<sub>3</sub> but the survival times were not statistically different. This suggests, but does not prove due to the variability between the trials, that background genes can influence the resistance or susceptibility to H5N1 HPAI virus challenge.

## CONCLUSION

We conclude that the B21 haplotype is not highly resistant to mortality of H5N1 HPAI virus following controlled challenge by a minimal chicken lethal dose of virus. Additional, genetic studies are necessary to determine the influence of epistatic genes on resistance or susceptibility to HPAI virus of these various MHC haplotype chicken lines.

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