

Gastrointestinal helminths in indigenous and exotic chickens in Vietnam: association of the intensity of infection with the Major Histocompatibility Complex

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SUMMARY

This study compared the prevalence and intensity of infections of helminths in 2 chicken breeds in Vietnam, the indigenous Ri and the exotic Luong Phuong. Also, possible correlations with the Major Histocompatibility Complex (MHC) were tested. The most prevalent helminths were *Ascaridia galli*, *Heterakis beramporia*, *Tetrameres mothedai*, *Capillaria obsignata*, *Raillietina echinobothrida* and *Raillietina tetragona*. Differences in prevalence and intensity of infection were found between the 2 breeds. Comparing the 2 groups of adult birds, Ri chickens were observed to have higher prevalence and infection intensities of several species of helminths, as well as a higher mean number of helminth species. In contrast, *A. galli* and *C. obsignata* were shown to be more prevalent in Luong Phuong chickens. Furthermore, an age-dependent difference was indicated in the group of Ri chickens in which the prevalence and the intensity of infection was higher for the adult than the young chickens for most helminths. The most notable exception was the significantly lower prevalence and intensities of *A. galli* in the group of adult chickens. In contrast, the prevalence and intensity were very similar in both age groups of Luong Phuong chickens. Using a genetic marker located in the MHC, a statistically significant correlation between several MHC haplotypes and the infection intensity of different helminth species was inferred. This is the first report of an association of MHC haplotype with the intensity of parasite infections in chickens.

Key words: helminths, *Tetrameres mothedai*, *Ascaridia galli*, chickens, indigenous, exotic, Vietnam, Major Histocompatibility Complex.

INTRODUCTION

In Vietnam, the total chicken population is estimated at approximately 160 million (FAOSTAT, 2005) with indigenous breeds constituting around 75% (Tran, 2002). In Northern Vietnam, the indigenous breed Ri is the most widespread breed, particularly among smallholder farmers. However, a Chinese commercial breed (Luong Phuong) is now being produced on governmental breeding farms and sold as day-old chicks. Due to favourable prices, fast growth and a plumage that resembles the popular Ri breed, the Luong Phuong breed is gaining significant

shares of the poultry production in Vietnam (personal observations). It is likely that, within populations of indigenous poultry breeds, some degree of natural resistance to diseases has developed through generations of exposure to different pathogens (Baelmans *et al.* 2005), and there is a considerable risk that local genetic resources associated with disease resistance will be lost in the course of introducing exotic breeds in an effort to increase production (Anderson, 2003; Mendelsohn, 2003).

Although viruses and bacteria may result in more dramatic outbreaks of disease, the importance of parasitic diseases should not be underestimated in poultry production, as the latter can be fatal to young and immunocompromised animals, and thus contribute significantly to mortality (Ikeme, 1971*a*; Soulsby, 1982). In addition, weight gain and egg production may be affected in adult and otherwise healthy birds (Ikeme, 1971*b*; Bhowmik *et al.* 1982).

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Only a few studies on the prevalence of helminth infections in scavenging chickens in Vietnam have been performed (Luong, 1997; Do *et al.* 1999). In these studies, up to 15 species of trematodes, 22 species of cestodes and 29 species of nematodes have been identified in local and exotic breeds.

Several studies have demonstrated that the Major Histocompatibility Complex (MHC) is involved in the genetic control of resistance to a range of viral or bacterial diseases, including Marek's disease (Rothschild *et al.* 2000; Plachy *et al.* 2003), Rous sarcoma virus (Plachy *et al.* 1992), avian leucosis (Yoo and Sheldon, 1992), fowl cholera (Lamont *et al.* 1987) and salmonellosis (Cotter *et al.* 1998; Liu *et al.* 2002). Furthermore, it has been shown that there is an association between the MHC and resistance to coccidiosis (Clare *et al.* 1985, 1989; Lillehoj *et al.* 1989; Caron *et al.* 1997). The genetic control of helminth infections in chickens is still poorly understood, and, to our knowledge, there is no information available on a link between the MHC and helminth infections in chickens. However, studies on sheep and cattle as well as mice have demonstrated associations between the MHC and resistance to helminths (Wassom *et al.* 1979; Stear *et al.* 1990, 1996; Wassom and Kelly, 1990; Behnke and Wahid, 1991; Outteridge *et al.* 1996).

The first aim of the present study was to compare the prevalence and intensity of infection of helminths in the 2 most widespread breeds of chicken in Northern Vietnam, the indigenous Ri and the exotic Luong Phuong. The second aim was to test for possible correlations between parasitological findings and MHC-haplotypes, as determined using a polymorphic microsatellite (LEI0258) which is located within the MHC-region on chromosome 16 (Fulton *et al.* 2006).

MATERIALS AND METHODS

Study area

Chickens were sampled from two neighbouring districts in Hanoi, Tu Liem and Đông Anh. Hanoi has a tropical climate (21°N, 106°E) with 4 seasons: May to September is the hot and rainy season, and November to March is the cold and dry season. In between are 2 transition seasons in April and October. The average temperature in the summer is 27.5 °C. June is the hottest month, with an average temperature of 29.2 °C. During winter the average temperature is 19.1 °C, with a temperature of only 17.2 °C in January. The monthly rainfall is on average 271 mm from May to September and 30 mm from November to March.

Study population

The study population consisted of chickens (*Gallus gallus*) of 2 breeds, an indigenous Vietnamese breed,

Ri, and Luong Phuong, a commercial exotic breed of Chinese origin. The chickens were purchased at a local market for small-holder farmers from the districts Tu Liem and Đông Anh in Hanoi, Vietnam. In order to minimize any bias caused by different levels of exposure, only chickens raised under comparable conditions with regards to flock size, farm-management and topography were selected for the study. According to interviews with the farmers at the market, all chickens were kept in semi-intensive outdoor production systems with access to small chicken houses. All chickens were fed with locally produced commercial feed supplemented with household wastes. Furthermore, only clinically healthy chickens were selected in an attempt to avoid possible bias caused by other diseases. Ten to 15 birds of each breed were purchased weekly during a period of 2 months (May and June, 2002), giving a total of 50 young (2 to 3 months old) and 50 adult (10 to 11 months old) chickens of each breed.

Post-mortem examinations and parasite identification

Each animal was examined according to the approach described by Permin and Hansen (1998). In short, the eyes were examined macroscopically, and the trachea washed and inspected for the presence of *Oxyspirura mansoni*. The crop and the proventriculus were examined for the presence of worms embedded under the serosal surface. The gastrointestinal tract was opened and the content sieved (63 µm). The intestinal mucosa was scraped to collect the remaining parasites embedded in these tissues. All collected material was examined under a stereomicroscope (20× magnification) to isolate the parasites. All helminths were counted and stored in 70% alcohol. All worms were identified using a light microscope (40× magnification). Before identifying the nematodes, they were cleared in lactic acid. All parasites were identified according to existing key descriptions (Soulsby, 1982; Anderson, 1992; Khalil *et al.* 1994; Permin and Hansen, 1998).

DNA isolation and MHC haplotyping

Before the post-mortem examination, 5 ml of EDTA-stabilized blood samples were taken from each chicken. DNA was isolated from 200 µl of packed blood cells using a salt/isopropanol protocol, as described previously (Juul-Madsen *et al.* 1993). The MHC haplotypes were determined by PCR-based genotyping of the LEI0258 microsatellite locus (Zoorob *et al.* 1998). The PCR amplification from genomic DNA was carried out in 25 µl reaction volumes using standard buffer (Amersham) containing 0.05 µM of each primer (the forward primer having been labelled with fluorescein), 0.4 mM of each dNTP, 1.5 mM MgCl₂ and 1 unit of *Taq* DNA polymerase (Amersham). After an initial 5 min of

denaturation at 94 °C, the amplification went through 25 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 2 min. The amplification was completed with a final extension for 10 min at 72 °C. The amplicons were measured by electrophoresis on a denaturing polyacrylamide gel in an ALF DNA sequencer (Amersham) to detect allelic polymorphism. A mixture of 10 fluorescein-labelled fragments of 50–500 bp (Amersham) was used as a size marker. Furthermore, DNA samples from 3 well-characterized lines of White Leghorn homozygous for the MHC haplotypes B15, B19 and B21 (Miller *et al.* 2004) were included on all gels as control samples. The different MHC haplotypes were finally classified according to the known repeat motif of the LEI0258 microsatellite (Fulton *et al.* 2006).

Statistical methods

A representative sample size for a cross-sectional study can be calculated by the formula $n = 1.96^2 pq / L^2$, where n = sample size, p = prevalence, $q = 1 - p$, and L is the limit of error on the prevalence (Martin *et al.* 1987). Not knowing the true prevalence of helminths in the populations included in the present study, we used an expected prevalence of 50%, as this requires the highest sample size (n). The maximum limit of error was 14%. As a result, the required sample size was calculated to be 49 but was, for convenience, set at 50.

The chi-square test was used to test for overall differences in parasite-specific prevalence among the 4 groups of chickens. Specific pairs of groups were tested using the Fisher's exact test, which calculates correct P -values. The D'Agostino-Pearson omnibus normality test was used to compare the distribution of intensity of infection with an expected Gaussian distribution, and differences in the median between the 4 groups were tested using the Kruskal-Wallis test with Dunn's post-test. All of the above-mentioned tests were performed using the statistical software package GraphPad Prism (GraphPad Software, 2005).

The analysis of the effect of MHC haplotypes on the intensity of infection was performed for the 6 most prevalent helminths (*Ascaridia galli*, *Heterakis beramporia*, *Tetrameres mothedai*, *Capillaria obsignata*, *Raillietina echinobothrida* and *Raillietina tetragona*) as follows. Firstly, for each helminth, the intensity of infection was categorized as zero, low, intermediate and high. The cut-off points used for defining the categories as low, intermediate and high intensity were distinct for the different helminths, and were chosen in such a way that the losses of the Kùlback-Leiber information about the counts were minimized (Table 3). Then, for each helminth, the association between the intensity of infection and the MHC haplotypes was studied by applying

a baseline-category logits model for multinomial distributed data (Agresti, 1990) using the zero-category as a reference. All of the analyses were adjusted for the effects of age and breed. The haplotypes entered in the model as a complete dominance effect. This procedure was applied to the 8 most frequent of the 19 haplotypes studied (see Fig. 1). Whether the haplotypes shown to be associated (significantly) with the intensity of infection for a particular helminth species contributed to resistance or susceptibility was evaluated with the Log (odds), in which $P(\text{level } x)/P(0)$ gives the probability with respect to the zero-category when the given haplotype occurs. Level x refers to 1 of the 3 categories low, intermediate or high infestation. Thus, values of Log (odds) of less than zero are interpreted to be associated with greater resistance in relation to a particular haplotype than the rest of the haplotypes. In contrast, values greater than zero are interpreted to be associated with greater susceptibility. All P -values were corrected for multiple testing by permutation procedures (Churchill and Doerge, 1994; Davison and Hinkley, 1997).

RESULTS

Parasitological findings

Of the 200 chickens, 191 (95.5%) were infected with 1 or more species of helminths. Of the young chickens, 90% of the Luong Phuong and 92% of the Ri were shown to harbour helminths, whereas all adult chickens of both breeds were infected. Seventeen different helminth species were isolated and identified (Table 1). Three different species of *Raillietina* were found, *R. echinobothrida*, *R. tetragona* and *R. cesticillus*. Due to their similarity in life-cycle, predilection site in the host etc, data were compiled for analyses. Also the data for the 2 species of *Echinostoma* found, *E. miyagawai* and *E. revolutum*, were compiled. The most prevalent cestodes were *Raillietina* spp. The most prevalent nematode species in all 4 groups were *Heterakis beramporia* and *Ascaridia galli*. Furthermore, *Capillaria obsignata* and *Tetrameres mothedai* were found to be highly prevalent in the groups of adult Luong Phuong and adult Ri chickens, respectively. Very few trematodes were found in the present study, with only adult Ri chickens having a noteworthy prevalence of *Echinostoma* spp. The chi-square test demonstrated significant differences between the 4 groups for the prevalence of all parasites, except for *Echinoparyphium recurvatum* and *Prosthogonimus cuneatus* (Table 1). As revealed by Fisher's exact test results, the difference could, in most cases, be attributed to a high prevalence in the group of adult Ri chickens. Thus, we showed that the prevalence of *Raillietina* spp., *S. cantaniana*, *O. mansoni*, *T. mothedai* and *Echinostoma* spp. was significantly higher in adult Ri compared with adult Luong Phuong, whereas the

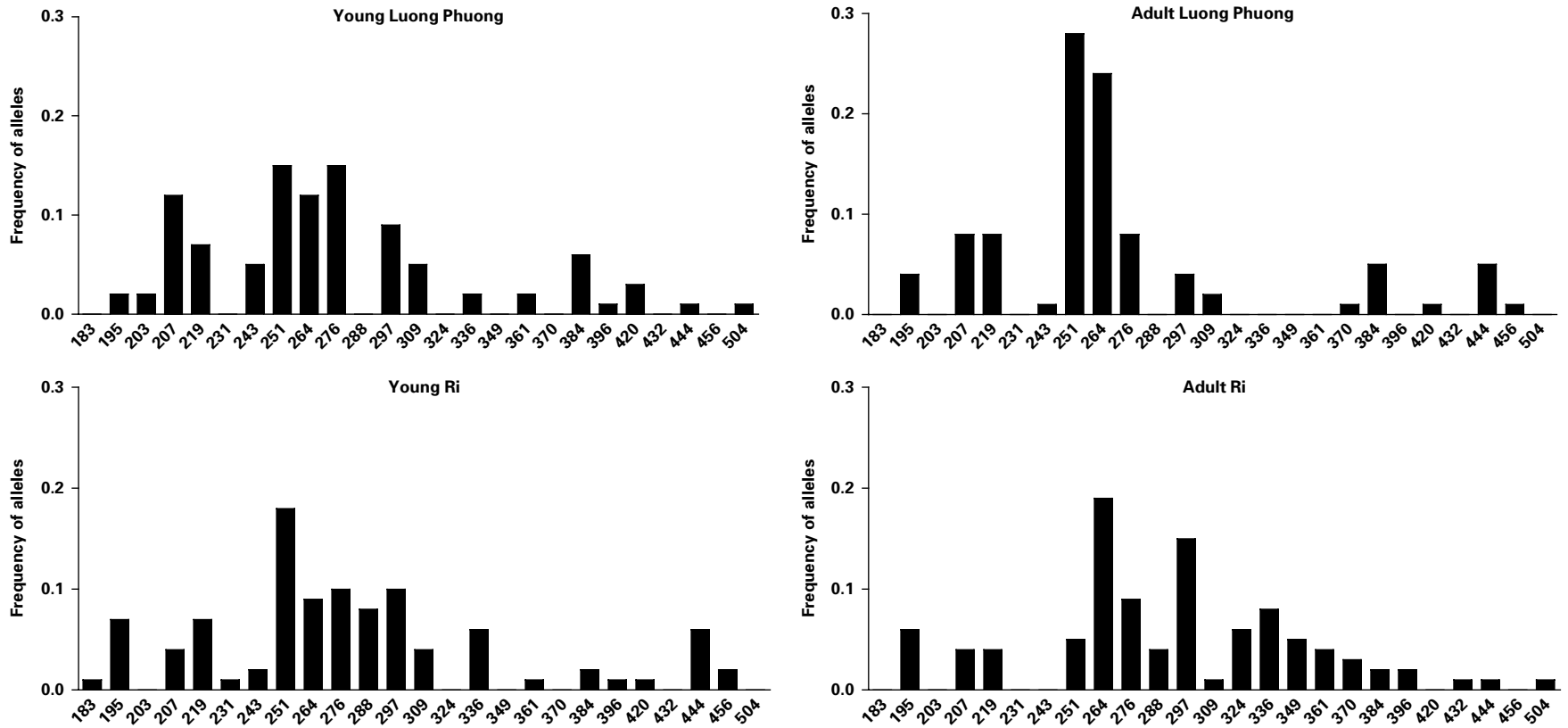


Fig. 1. Distributions of alleles of the MHC-situated LEI0258-microsatellite found in 4 populations of chickens in Hanoi, Vietnam. The alleles were classified according to variation in the size (base pairs). Fifty chickens from each population were examined and each graph thus presents the frequency of 100 alleles.

Table 1. Prevalence of helminths in young and adult chickens of two breeds (Ri and Luong Phuong) in the Tu Liem and Đông Anh districts of Hanoi, Vietnam (2002)

Helminth species	Luong Phuong				Ri				X ² -test (P) ¹	Fisher's exact test ²
	Young		Adult		Young		Adult			
	Prevalence (%)	95% CI	Prevalence (%)	95% CI	Prevalence (%)	95% CI	Prevalence (%)	95% CI		
Cestodes										
<i>Orientolepis exigua</i>	2	0–12	4	0·4–14	0	0–9	18	10–31	***	b
<i>Railletina</i> spp.	46	33–60	56	43–69	24	14–38	88	76–95	***	b c
<i>Staphylepis cantianiana</i>	0	0–9	0	0–9	0	0–9	26	16–40	***	b c
Nematodes										
<i>Acuaria hamulosa</i>	0	0–9	0	0–9	0	0–9	10	4–22	**	
<i>Ascaridia galli</i>	58	44–71	76	63–86	68	54–79	44	31–58	**	b c
<i>Capillaria obsignata</i>	14	7–27	64	50–76	6	1–2	30	19–44	***	a b c
<i>Dispharynx nasuta</i>	0	0–9	2	0–12	0	0–9	8	3–19	*	
<i>Eucoleus annulatus</i>	0	0–9	0	0–9	0	0–9	8	3–19	**	
<i>Heterakis beramporia</i>	54	35–62	82	69–91	52	39–65	82	69–91	***	a b
<i>Oxyspirura mansoni</i>	2	0–12	6	1–2	0	0–9	54	40–67	***	b c
<i>Tetrameres mothedai</i>	8	3–19	4	0·4–14	0	0–9	58	44–71	***	b c
Trematodes										
<i>Echinoparyphium recurvatum</i>	0	0–9	0	0–9	0	0–9	2	0–12	n.s.	
<i>Echinostoma</i> spp.	2	0–12	0	0–9	0	0–9	40	28–54	***	b c
<i>Prosthogonimus cuneatus</i>	2	0–12	0	0–9	0	0–9	4	0·4–14	n.s.	

¹ * = 0·01 < P < 0·05; ** = 0·001 < P < 0·01; *** = P < 0·001.

² The letters a, b, c and d indicates statistically significant differences (P < 0·05) between specific pairs of groups as follows:

a = Young Luong Phuong × Adult Luong Phuong; b = Young Ri × Adult Ri;

c = Adult Luong Phuong × Adult Ri; d = Young Luong Phuong × Young Ri.

prevalence of *A. galli* and *C. obsignata* was shown to be significantly lower in adult Ri compared with adult Luong Phuong. When comparing young and adult Ri, the prevalences of *O. exigua*, *Raillietina* spp., *S. cantaniana*, *C. obsignata*, *H. beramporia*, *O. mansoni*, *T. mothedai* and *Echinostoma* spp. were calculated to be significantly higher in the group of adult Ri, whereas *A. galli* was shown to be more prevalent in the group of young Ri chickens. When comparing young and adult Luong Phuong, the prevalence of *C. obsignata* and *H. beramporia* was significantly higher in the group of adult chickens. The only statistically significant difference between the young chickens of the 2 breeds was in the prevalence of *R. echinobothrida*, which was found to be higher in young Luong Phuong chickens.

In all 4 groups, *A. galli* and *H. beramporia* constituted the majority of the worm burden, followed by *Raillietina* spp. and *C. obsignata*. A relatively high mean intensity of infection of *T. mothedai* was also found in the group of adult Ri chickens (Table 2). The intensities of infection for all parasite species did not show a normal distribution, and the non-parametric Kruskal-Wallis test was therefore used to compare the medians for the 4 groups (Table 2). Statistically significant differences between the 4 groups for all parasites, except for *E. recurvatum* and *P. cuneatus*, were found. Furthermore, Dunn's post-test showed that the difference in most cases involved the group of adult Ri chickens. This group had the highest intensity of infection for all parasites, except for *A. galli* and *C. obsignata*, of which adult Luong Phuong were found to harbour significantly more worms. Statistically significant differences were also found between young and adult Luong Phuong chickens for *A. galli*, *C. obsignata* and *H. beramporia*.

Adult chickens of both breeds harboured a significantly greater number of helminth species compared with the respective groups of young chickens ($P < 0.001$). Adult Ri harboured significantly more species than adult Luong Phuong ($P < 0.001$), whereas no difference was found between the 2 groups of young chickens.

Distribution of MHC haplotypes

The distributions of alleles for each of the 4 populations are shown in Fig. 1. A total of 19 different alleles were found for the Luong Phuong chickens, with 17 and 14 different alleles being recorded for the group of young and adult chickens, respectively. In the group of Ri chickens, 24 different alleles were detected, with 19 alleles being recorded in each age group. Visual evaluation indicates an apparent difference in allelic distribution between young and adult Luong Phuong chickens, whereas for the 2 groups of Ri chickens the distribution appeared to be similar.

Analysis using the Fisher's exact test revealed a statistically significant lower frequency of the 251 bp allele in the group of adult Ri chickens. In contrast, the frequency of the 264 bp allele was higher (although not significant) in the adult group ($P = 0.065$). For the Luong Phuong chickens, statistically significant higher frequencies of the 251 bp and 264 bp alleles were recorded for the group of adult chickens when compared with the young group.

A total of 9 and 13 of the 100 Luong Phuong and Ri chickens, respectively, was shown to be homozygous at the LEI0258 locus. Analysis using the Fisher's exact test showed that the difference was not statistically significant ($P = 0.499$).

Correlation between worm burdens and MHC haplotypes

The effect of the MHC-haplotype on the intensity of infection with helminths was examined (Table 3). It was found with statistical significance that the 276 bp allele was associated with the intensities of 3 (*A. galli*, *H. beramporia* and *T. mothedai*) of the 6 helminths included in the test. No association was detected between any haplotype and the compiled intensities for the 3 *Raillietina* spp. found, but the 251 bp and 264 bp alleles were shown to have a marked but insignificant association with the intensity of *R. tetragona* infection. From the Log (odds), it was possible to evaluate whether these haplotypes were associated with resistance or susceptibility to a particular helminth (Table 4). Thus, the 276 bp allele was inferred to be associated with resistance against *A. galli*, *H. beramporia* and *T. mothedai*. In contrast, the 251 bp and 264 bp alleles were inferred to be associated with increased susceptibility to *R. tetragona*.

DISCUSSION

Prevalence of helminths

The high overall prevalence of helminth parasites found in the present study is in accordance with several investigations from Africa (Ssenyonga, 1982; Permin *et al.* 1997; Poulsen *et al.* 2000; Irungu *et al.* 2004), as well as Asia, i.e. Pakistan (Bilqees and Khan, 1985), India (Yadav and Tandon, 1991) and Vietnam (Luong, 1997). The latter study was performed in the South of Vietnam, and identified 66 species of helminths in 832 chickens. In contrast, a study performed on 511 chickens in Hanoi in the North of Vietnam revealed only 5 species of helminths and at a lower prevalence (Do *et al.* 1999). The present study, which was performed in the same districts of Hanoi as the study by Do *et al.* (1999), revealed 17 different helminth species with prevalences similar to those reported in an investigation by Luong (1997). This information suggests that the

Table 2. Mean worm burdens with standard error of mean (S.E.M.) and variance in young and adult chickens of two breeds (Ri and Luong Phuong) in the Tu Liem and Đông Anh districts of Hanoi, Vietnam (2002)

(N=50 for all 4 groups.)

Helminth species	Young Luong Phuong			Adult Luong Phuong			Young Ri			Adult Ri			Statistical tests				
	Mean	S.E.M.	Var.	Mean	S.E.M.	Var.	Mean	S.E.M.	Var.	Mean	S.E.M.	Var.	Kruskal-Wallis ¹	Dunn's post test ²			
														a	b	c	d
Cestodes																	
<i>Orientolepis exigua</i>	0.08	0.08	0.32	0.12	0.10	0.52	0.00	0.00	0.00	0.82	0.29	4.11	***		+	+	
<i>Railletina</i> spp.	2.58	0.65	21.45	3.36	0.78	30.40	1.12	0.34	5.66	8.12	1.02	51.54	***		+	+	
<i>Staphylepis cantaniana</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.54	0.44	9.72	***		+	+	
Nematodes																	
<i>Acuaria hamulosa</i>	0.00	0.00	0.00	0.00	0.00	208.12	0.00	0.00	0.00	0.40	0.24	2.94	**		+	+	
<i>Ascaridia galli</i>	3.24	0.67	22.10	10.20	2.04	45.13	7.96	1.78	157.59	4.18	1.02	52.31	**	+		+	
<i>Capillaria obsignata</i>	0.96	0.47	10.81	3.88	0.95	0.02	0.14	0.10	0.53	1.22	0.40	8.05	***	+	+	+	
<i>Dispharynx nasuta</i>	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.56	0.32	5.15	*		+		
<i>Eucoleus annulatus</i>	0.00	0.00	0.00	0.00	0.00	107.79	0.00	0.00	0.00	0.28	0.18	1.59	**		+	+	
<i>Heterakis beramporia</i>	7.50	2.03	206.91	9.08	1.47	0.75	1.90	0.50	12.70	12.14	1.74	150.69	***	+	+		
<i>Oxyspirura mansoni</i>	0.06	0.06	0.18	0.16	0.12	2.53	0.00	0.00	0.00	2.14	0.43	9.10	***		+	+	
<i>Tetrameres mothedai</i>	0.24	0.18	1.66	0.26	0.22	208.12	0.00	0.00	0.00	3.68	0.62	19.32	***		+	+	
Trematodes																	
<i>Echinoparyphium recurvatum</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.12	0.72	n.s.				
<i>Echinostoma</i> spp.	0.12	0.12	0.72	0.00	0.00	0.00	0.00	0.00	0.00	1.36	0.34	5.91	***		+	+	
<i>Prosthogonimus cuneatus</i>	0.04	0.04	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.08	0.34	n.s.				

¹ * = 0.01 < P < 0.05; ** = 0.001 < P < 0.01; *** = P < 0.001.² Dunn's post test was performed to compare specific pairs of groups as follows: a = Young Luong Phuong × Adult Luong Phuong; b = Young Ri × Adult Ri; c = Adult Luong Phuong × Adult Ri; d = Young Luong Phuong × Young Ri; + indicates statistically significant differences (P < 0.05).

Table 3. P-values adjusted for multiple testing (based on 10 000 shuffles)

Helminth Species	Max. information	Cut-points		Haplotypes			
				251	264	276	
<i>R. echinobothrida</i>	0.74	0	2	6	0.0825	0.0721	—
<i>R. tetragona</i>	0.70	0	2	5	—	—	—
<i>A. galli</i>	0.96	0	3	10	—	—	0.0082
<i>C. obsignata</i>	0.66	0	1	6	—	—	—
<i>H. beramporia</i>	0.99	0	3	11	—	—	0.0047
<i>T. mothedai</i>	0.47	0	3	7	—	—	0.0308

Table 4. Resistance or susceptibility to helminths, given as Log (odds), of MHC haplotypes found to be associated with worm burden (See Table 3)

(Values less than zero are associated with greater resistance of a given haplotype than the rest of the haplotypes found in the present study. In contrast, values of greater than zero are associated with greater susceptibility. All were significant except when marked with a *.)

Category	276	276	276	251	264
	<i>Heterakis beramporia</i>	<i>Ascaridia Galli</i>	<i>Tetrameres mothedai</i>	<i>Raillietina tetragona</i>	<i>Raillietina tetragona</i>
0	—	—	—	—	—
Low	-2.71	-1.35	-18.54	1.44	1.00
Intermediate	-1.29	-1.79	-21.62	0.50	0.66
High	-0.22	-1.42	2.11*	2.39	0.81

apparent discrepancy between these studies is due to year-to-year variation rather than geographical, ecological and/or climatic differences between the North and South of Vietnam. The present study, together with the 2 above-mentioned studies, suggest that the most prevalent helminth species in Vietnam are *A. galli*, *H. beramporia*, *T. mothedai* and *Raillietina* spp. Also, this study showed a high prevalence of *C. obsignata* in the adults of both the Ri and the Luong Phuong breeds. With the exception of *H. beramporia*, these parasites are all proposed to cause production losses either directly or indirectly via an interaction with other pathogens (Ruff, 1999; Ruff and Norton, 2003; McDougald, 2003). *H. beramporia*, like *Heterakis gallinarum*, is probably a possible carrier of the protozoan *Histomonas meleagridis*, which causes the economically important disease ‘blackhead’ (enterohepatitis) in turkeys and chickens (Ruff and Norton, 2003). Therefore, this parasite could be an important risk to Vietnamese poultry production, although to our knowledge, no information is presently available on the prevalence of *H. meleagridis* in Vietnam.

Effect of age on prevalence and intensity of infection

In humans it has been found that most helminths usually follow the same epidemiological pattern. Their prevalence reaches a peak in childhood, which remains stable throughout adulthood, whereas the

intensity of infection (worm burden/parasite egg excretion) decreases to lower levels during adulthood, after having peaked in early childhood (Behnke, 1987; Bundy, 1988; Turner *et al.* 2003; Hayes *et al.* 2004). The influence of the age of the chickens on the prevalence and intensity of helminth infections has only been examined in relatively few studies, but some results suggest a similar trend in chickens. Magwisha *et al.* (2002) concluded that young chickens (12–24 weeks of age) carried heavier worm burdens than adults. In the present study, the prevalence of all but 2 parasites (*C. obsignata* and *H. beramporia*) was similar in both age groups of Luong Phuong chickens, and we did not observe an elevated intensity in the group of young chickens. In the group of Ri chickens, the prevalence and intensity of infection were higher for the adult than the young chickens for most of the helminths recorded. As reviewed by Woolhouse (1998), field studies of human helminthoses have frequently reported differences between populations in the age at which the intensity of infection peaks. It has been argued that this ‘peak shift’ is primarily due to different levels of exposure. Thus, populations subjected to low levels of exposure will have lower peak levels of infection at an older age (Woolhouse, 1998). Therefore, it is possible that the chickens included in the present study did not follow the typical epidemiological pattern due to a relatively low exposure to helminth infection. Compared with previous studies

(Yadav and Tandon, 1991; Permin *et al.* 1997, 2002; Poulsen *et al.* 2000; Magwisha *et al.* 2002), the intensity of infection (worm burdens and mean number of helminth species) was, for most of the species recorded, shown to be lower in the present study. This may be due to differences in the exposure to helminths, which may simply reflect lower prevalences in the study area at the time of sampling. However, differences between production systems may also relate to different levels of exposure to helminths (Permin *et al.* 1999). The chickens included herein were thus kept in semi-intensive production systems and fed commercial feed, whereas the chickens in previous prevalence studies were scavenging freely around the households, having access to little or no supplementary feed.

The most notable exception to the higher prevalences demonstrated for most of the helminths in the group of adult Ri chickens, was the significantly lower prevalence of *A. galli*. Similarly, the adult Ri chickens were found to harbour a considerably lower mean number of *A. galli* (adult Ri: 4.2; young Ri: 8.0), although the difference was not statistically significant. It is possible that this trend is simply due to a higher exposure of the chickens to this parasite, which then causes the typical epidemiological pattern of a lower intensity among the adults. However, as this pattern was not observed for the Luong Phuong chickens, it is also possible that genetic factors are involved (discussed below). Results from several studies have suggested that resistance to *A. galli* increases with age, regardless of previous exposure to the parasite (Ackert *et al.* 1935; Tongson and McCraw, 1967; Ikeme, 1973; Gauly *et al.* 2005). In contrast, a study by Idi *et al.* (2004) concluded that the age of chickens only partially influences resistance to *A. galli* infection. However, except for the study by Gauly *et al.* (2005), the chickens used in these studies ranged from 2 to 16 weeks of age, and, therefore, the results are not directly comparable. Gauly *et al.* (2005) showed that chickens at the age of 12 or 18 weeks had higher worm burdens as well as higher faecal egg excretion than chickens of the age of 6 or 24 weeks. The authors concluded that, rather than age *per se*, the hormonal and immune status, related to the point of lay, caused the increased susceptibility. The point of lay in Ri chickens is around 26 weeks of age. Thus, it could be speculated that the high intensity of helminths recorded in the adult group might result from a period of increased susceptibility around this time.

Effect of breed on prevalence and intensity of infection

In the present study, we compared the prevalence and intensity of infection in 2 age groups of 2 different chicken breeds, and significant differences

were found between the 4 groups for almost all parasites. These differences could, in most cases, be attributed to high prevalences and intensity of infections in the group of adult Ri chickens. Thus, the group of adult Ri was observed to have not only a higher prevalence of several species of helminths but also to harbour a higher mean number of helminth species than the group of adult Luong Phuong chickens. In contrast, *A. galli* and *C. obsignata* were found to be more prevalent among adult birds of the Luong Phuong breed than the adult Ri chickens. Similarly, the adult Ri chickens were found to harbour the highest worm burden for most of the parasite species, with the exception that the adult Luong Phuong harboured significantly more worms of *A. galli* and *C. obsignata*. These observations are in accordance with another investigation from Vietnam, which concluded that the 3 cestode species recorded (*R. echinobothrida*, *R. tetragona*, *R. cesticillus*) were more prevalent in indigenous Ri chickens than in Leghorn chickens and that the 2 nematode species detected (*A. galli* and *H. gallinarum*) were more prevalent in Leghorn chickens than in Ri chickens (Do *et al.* 1999). Although the Ri chickens in the present study seemed less susceptible to *A. galli* and *C. obsignata*, it is possible that the lower prevalences and intensities of infection with these species is a result of an increased competition in chickens which are heavily infected with other helminth species. However, no correlation was found between total worm burdens and the number of *A. galli* and *C. obsignata*, thus suggesting that the Ri chickens are less susceptible to infection with these particular helminths than the Luong Phuong chickens. Furthermore, an experimental *A. galli* infection study performed on-farm in Vietnam on the same breeds confirmed that Ri chickens are less susceptible to *A. galli* (unpublished results).

It has been argued that current commercial breeds are particularly susceptible to disease as selection for disease resistance, if any, is fortuitous due to the focus of the poultry industry on breeding for production traits (Bumstead, 1996; Ruff, 1999). Furthermore, genetic selection towards improved production traits has been shown to reduce the performance of the adaptive immune system (Qureshi and Havenstein, 1994; Cheema *et al.* 2003). In contrast, it is likely that some degree of natural resistance to diseases has developed within populations of indigenous poultry breeds through generations of exposure to pathogens. Interestingly, the present study revealed the indigenous Ri breed to be more susceptible to helminths (except for *A. galli* and *C. obsignata*) than the commercial Luong Phuong breed; Do *et al.* (1999) reported similar findings. As selection of chickens in our study was based on short interviews with the farmers at the market, it was not possible to obtain a completely objective evaluation of farm conditions. Therefore, it is possible that the

results were affected by farm-to-farm variation. However, to the best of our knowledge, the chickens in the present study came from comparable farms with regards to flock size, farm-management and –topography, which should reduce the risk that the results were biased by a skewed exposure to helminths or other diseases likely to affect the susceptibility of the chickens. Furthermore, other studies also suggest that commercial breeds are less susceptible to helminth infections than outbred populations. Accordingly, Permin and Ranvig (2001) showed the commercial breed Lohmann Brown to be less susceptible to an experimental infection with *A. galli* than the indigenous Danish Landrace. Similarly, another study found that New Hampshire chickens were less susceptible to *A. galli* than Danish Skalborg chickens (Schou *et al.* 2003). In addition, unpublished results from a study in Bangladesh found that the indigenous Deshi breed were more susceptible to helminth infections than the Sonali breed (a cross of male Rhode Island Red and female Fayoumi). Although these studies were performed on different breeds and under different conditions, they all indicate that it would be wrong to presume that indigenous breeds are less susceptible to helminth infections than commercial breeds. It is interesting that the indigenous chickens included herein seemed less susceptible to *A. galli*, whereas the opposite was the case in the studies by Permin and Ranvig (2001) and Schou *et al.* (2003). This dissimilarity emphasizes the complexity of resistance/susceptibility to helminth infections and the importance of using several pathogens when examining the disease resistance potential in different breeds.

Association of Major Histocompatibility Complex with intensity of helminth infections

Compared with indigenous breeds, high levels of selection for a few economically important traits have decreased the genetic diversity, in general, in commercial breeds. In contrast, indigenous breeds show high levels of phenotypic variability and increased fitness under natural conditions (Giovambattista *et al.* 2001). The MHC is the most polymorphic gene cluster known, and it is very likely that infectious diseases have been the main selection force (Jeffery and Bangham, 2000). However, at most neutrally evolving loci, polymorphism will be reduced due to genetic drift (Hughes, 2002), and, as little or no selection for MHC-mediated disease resistance occurs in commercial breeds, it is therefore likely that MHC polymorphism is reduced in commercial breeds compared to outbred populations. Therefore, it is not surprising that we found 26% more alleles of the LEI0258 microsatellite among the Ri chickens than among the Luong Phuong chickens. Nonetheless, the number ($n=19$) of alleles found in the

group of Luong Phuong seems remarkably high compared with other commercial breeds. In a commercial Lohman Silver line ($n=79$) only 3 different alleles were found (Fink *et al.* 2005). Five different alleles were found in a Lohman Brown line ($n=75$) and 7 different alleles in 75 chickens of a Skalborg \times New Hampshire cross (unpublished results). As reviewed by Wegner *et al.* (2004) MHC heterozygosity seems to be advantageous in MHC-mediated disease resistance due to the increased diversity of antigens capable of being presented to T cells. Therefore, the frequency of heterozygosity at the MHC is probably higher in outbred populations exposed to all kinds of infectious agents, whereas MHC heterozygosity would be expected to be lower in commercial populations due to a lack of such exposure in the non-infected environment that the elite birds of the Luong Phuong breed is derived from. A higher frequency of individuals heterozygous at the LEI0258 locus in the Ri chickens compared with the Luong Phuong chickens would be expected. However, no statistically significant difference between these 2 breeds was detected. The present results therefore indicate that, although somewhat reduced, MHC polymorphism is relatively intact in the Luong Phuong chickens compared with other commercial breeds.

The apparent difference in allelic distributions between young and adult Luong Phuong chickens was in contrast to the more similar distributions in the 2 groups of Ri chickens. We also calculated a statistically significant difference between the 2 age groups of Ri chickens for the 251 bp allele and for the 251 bp and 264 bp alleles between the 2 age groups of Luong Phuong chickens. However, these differences are unlikely to have been caused by infection with helminths, as they remain in the environment at a relatively constant level. A more likely explanation is that epidemic outbreaks with viral or bacterial pathogens have caused these differences. However, it is also possible that due to the high number of alleles present in these populations, larger groups of chickens would have given a more representative section and distribution of haplotypes.

An effect of MHC haplotypes on intensity of infection with several helminths species was inferred. The 276 bp allele was found to be of particular interest, as chickens bearing this allele seemed more resistant to *H. beramporia*, *A. galli* and *T. mothedai* in terms of having significantly lower worm burdens compared with chickens bearing other alleles. In contrast, the 251 bp and 264 bp alleles were associated with increased susceptibility to *R. tetragona*. This is the first report of an association between MHC haplotype and worm burden in chickens. In sheep, cattle and mice, the MHC has been shown to be associated with several parasite species (Wassom *et al.* 1979; Stear *et al.* 1990, 1996; Wassom and Kelly, 1990; Behnke and Wahid, 1991; Outteridge

et al. 1996). However, genes outside of the MHC also appear to influence susceptibility to parasites (Wassom and Kelly, 1990; Behnke *et al.* 2003) and the effect of the MHC in the present study may therefore partly have been overshadowed by the effect of background genes. In an experimental set-up, this problem can be overcome by developing congenic strains which share common sets of background genes but differ in the MHC (Wassom and Kelly, 1990).

In the present study, the LEI0258 microsatellite was used as a marker of the MHC. It is not possible to be absolutely certain that chickens with the same LEI0258 alleles have the exact same genetic composition of the MHC, as this would require the sequencing of the whole complex. However, recombination within the MHC occurs at a very low frequency in chickens (Plachy *et al.* 1992). Therefore, we consider that within each of the 2 breeds, all chickens are at the same linkage phase and that the LEI0258 microsatellite locus is a reliable marker for a primary classification.

In conclusion, the results of the present study suggest that the most prevalent helminth species in Vietnam are *A. galli*, *H. beramporia*, *T. mothedai*, *C. obsignata* and *Raillietina* spp. Differences in prevalence as well as intensity of infections were found between the 2 breeds. Comparing the 2 groups of adult birds, the indigenous Ri chickens were observed to have higher prevalences and intensities of infection for several species of helminths, as well as a higher mean number of helminth species than the commercial Luong Phuong chickens. In contrast, *A. galli* and *C. obsignata* were found to be more prevalent in the Luong Phuong than the Ri chickens. Furthermore, an age-dependent difference was found in the group of Ri chickens in which the prevalence and the intensity of infection were higher among the adult than the young chickens for most of the helminths recorded. The most notable exception to this was the significantly lower prevalence and worm burdens of *A. galli* in the group of adult chickens. In contrast, the prevalence and intensity were very similar for both age groups of Luong Phuong chickens. Using a genetic marker in the MHC, we found a higher degree of polymorphism among the Luong Phuong chickens than normally expected for a commercial breed. Furthermore, we found a statistically significant correlation between several MHC haplotypes and intensity of infection for different helminth species.

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