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**Prediction of Immunocompetence and Resistance
to Disease by Using Molecular Markers of the Major
Histocompatibility Complex**

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1. ABSTRACT

This project utilized two live-animal populations in an integrated research program to identify molecular markers for immune response and disease resistance. The populations each had their foundation from meat-type commercial breeder chicken lines of their respective countries. Investigations effectively used unique availability of resources in each country to study commercial-type environments in Israel and line-crosses with diverse inbred lines in the US. Two bacterial systems were investigated to cover both respiratory and gastrointestinal, and primary and secondary, infections. Individual experimental groups of animals were evaluated for combinations of vaccine antibody levels, response to pathogen challenge, growth parameters, genetic background and molecular markers. The positive association of antibody level with resistance to disease was confirmed. Effectiveness of genetic selection for vaccine antibody response level was demonstrated. Molecular markers, both inside and outside the MHC region, were associated with antibody response and resistance to disease. Markers were shown to have a generalized effect, by association with multiple traits of immune response and disease resistance. The impact of genetic background on marker effect was shown to be important. The overall results demonstrate the effectiveness of selection on vaccine antibody response and the potential of molecular marker-assisted selection to improve efficiency of production of meat-type chickens by reducing genetic susceptibility to disease.

2. OBJECTIVES OF THE ORIGINAL RESEARCH PROJECT

1. Identify class II MHC DNA markers associated with immune response in meat-type chickens.
2. Identify class II and IV MHC DNA markers associated with disease resistance in meat-type chickens.
3. Test the validity of using class II and IV MHC DNA markers for improving immune response and disease resistance in diverse commercial genetic stocks.

Because of research progress by the investigators, and advances in relevant biotechnologies in the scientific community, the research achievements of this BARD project surpassed the original proposed objectives, while remaining true to the major goal of the project to investigate molecular markers of immunocompetence and disease resistance. Evidence suggested that the originally proposed genetic system, the *MHC*

class II and IV, plays a small role in the investigated trait. Therefore, other MHC-linked RFLP markers, and a set of microsatellite primers spread over the genome, were acquired for evaluation as additional potential molecular marker systems for immune response and disease resistance. These advances led to a more comprehensive overall project.

3. RELEVANT DATA, METHODOLOGY, RESULTS AND DISCUSSION

3.1 Studies with Israeli Chicken Lines that were Divergently Selected for High or Low Antibody Response to Early *E. coli* Vaccination

3.1.1 Antibody Response and Mortality Under Controlled and Farm Conditions

3.1.1.1 Relating antibody response and mortality under experimental and farm conditions.

The objectives of this BARD project had been to identify DNA markers associated with quantitative trait loci (QTL) affecting immune response and disease resistance in meat-type chickens, and to test the effectiveness of these markers for improving immune response and disease resistance in diverse commercial genetic stocks. Thus, the project's ultimate goal has been to genetically improve immunocompetence and viability of commercial broilers. The Israeli resource population, used to detect association between these traits and DNA markers consisted of F₂ and backcross (BC) progeny from a cross between two lines divergently selected, since 1987, for high (HH) or low (LL) antibody (Ab) response to *Escherichia coli* (*E. coli* or *EC*) vaccination at 10 days of age (Leitner *et al.*, 1992; Heller *et al.*, 1992). Successful detection of marker-QTL depends on the genetic variation in the resource populations, that reflects the magnitude of genetic divergence between the selected lines. Therefore these lines had been continuously selected during the project's years, and indeed the divergence between the lines in Ab response to *E. coli* vaccination increased from 2.5-fold to 4.5-fold in generations S₅ and S₉, respectively (Yonash *et al.*, 1996). Moreover, the same study revealed substantial genetic variation in Ab to *E. coli* within the lines at S₉ (heritability estimates of 0.2 to 0.4, Yonash *et al.*, 1996). With such heritability, the continued selection in the HH and LL lines (generation S₁₂ hatched in 1998) has further increased the divergence between the lines, and therefore more efficient detection of marker-QTL association is expected in

resource populations derived from HHxLL cross at later generations (S_{11}) than the cross at S_5 .

However, the lines had been selected on Ab response to a single antigen, their growth rate is lower than current commercial broilers, and their immunocompetence and viability had never been tested under commercial farm conditions. Therefore, it was necessary to establish the practical relevance of the genetic differences between the HH and LL lines. The following aspects had to be investigated:

- (a) Effect of Ab response to *E. coli* vaccination on survival to pathogenic *E. coli* challenge.

Previous studies demonstrated that survival rate was higher in the HH line than the LL line (Leitner *et al.*, 1992; Yonash *et al.*, 1994). However, in these studies the birds were challenged by injection of pathogenic *E. coli* that led to mortality rates of 40 to 70%, and the lines differed only when the birds were vaccinated against *E. coli* 10 days earlier. Since both conditions, the method of challenge and the pre-vaccination against *E. coli*, do not resemble natural *E. coli* exposure of commercial broilers, a more realistic method of challenge had to be developed.

- (b) Association between Ab response to *E. coli* vaccination and natural exposure to *E. coli*.

The entire selection of the HH and LL lines, as well as the Ab responses measured in the resource population, had all been done following controlled immunization with an *E. coli* vaccine, or recently following a controlled tracheal administration of pathogenic *E. coli*. Therefore it has been essential to determine the level of agreement between Ab response to artificial controlled exposure to *E. coli*, and the Ab response to natural exposure to *E. coli* in commercial farms.

- (c) Association between Ab response to *E. coli* and response to commercial vaccination.

The project was aiming at genetic improvement of overall immunocompetence and disease resistance of broilers. Therefore, in addition to measuring Ab response to *E. coli*, it was important to determine the differences

between the HH and LL lines in their Ab response to standard vaccination programs, and in viability, under commercial farm conditions.

- (d) Association between Ab response to vaccination and growth rate of the broilers.

Since the HH and LL lines were not selected for body weight since 1987, their growth rate is substantially lower than that of current commercial broilers. In view of reports suggesting that rapid growth rate could be associated with lower immune response and viability, the selected lines (HH and LL) and their crosses (HHxLL, comparable to the resource population used in this project to detect marker-QTL associations) had to be compared with fast-growing commercial broilers for their Ab response as well as survival to challenge with pathogenic bacteria. These comparison had to be conducted in controlled experimental conditions and, mainly, in commercial farms.

3.1.1.2 Antibody response, growth rate and mortality under farm conditions.

Trials under farm conditions were conducted in 1996, 1997 and 1998. All of them included commercial broilers (designated CC), the HH and LL lines, and progeny of matings between commercial (CC) females and males from the HH or LL line; the progeny groups were designated HC and LC, respectively. A preliminary trial under semi-commercial conditions was conducted in early 1996 and was described in detail in the 2nd annual report. A more comprehensive field trial was conducted early 1997 and reported in the 3rd annual report. A similar trial was conducted also in 1998, and it included also a sixth group, the F₄ generation of a HHxLL cross, designated HL. Thorough description of the trials conducted in 1997 and 1998, and discussion of their results, are given in the attached manuscript titled "Immunocompetence and viability under commercial conditions of broiler groups differing in growth rate and antibody response to *E. coli* vaccine" by Yunis, Ben-David, Heller and Cahaner.

In summary, mean Ab levels of the six groups fitted perfectly to the additive genetic model, and were independent of the groups' different growth rates. These results, with excellent agreement between the two years, clearly indicate that the HH and LL lines, and their cross (i.e., the resource population we had used) yield Ab data that are relevant also to commercial broilers and to

farm conditions. As for mortality, its rate was similarly low in all three slow-growing groups (HH, LL and HL) despite the large differences in their Ab levels, both for *E. coli* and NDV. Growth rate of these groups was 33% lower than that of the commercial broilers. Whereas the CC broilers suffered high mortality, apparently due to their rapid growth rate, it appears that the birds in all three slow-growing groups were similarly resistant to mortality due to infectious diseases. Consequently, differences in immunocompetence between the HH and LL lines were not reflected in mortality. In other words, these lines, and the crosses between them (HL chicks), do not facilitate detection of relevant genetic variation in disease resistance and overall viability. This conclusion also explains the results of the controlled-challenge trials, where HH birds did not exhibit lower mortality despite their higher Ab responses. As expected, growth rate of the HC and LC groups averaged only 17% less than CC, and their average mortality was also intermediate between the commercial broilers and the three slow-growing groups. However, average mortality of LC birds was significantly higher than that of HC birds in both years, suggesting a negative correlation between mortality and Ab levels in these groups. It appears that the birds in these crosses grew fast enough to express genetic association between Ab response and viability. Therefore detection of markers for viability, and molecular dissection of genetic correlation between immune response, viability and growth rate, should be conducted in populations where the genetic potential for growth approaches that of current commercial broilers.

3.1.2 Candidate Genes: MHC-Related RFLP Markers and Antibody Response

3.1.2.1 Phase 1: Genotyping a resource population of F_2+BC_1 with an MHC class IV probe.

Phase 1 of the search for association between antibody response and MHC-related RFLP marker was conducted in a resource population consisting of about 160 F_2 birds and 160 backcross (BC) birds, all progeny of a few F_1 males obtained from a HHxLL cross in the S5 generation. These males were mated with their F_1 female counterparts to produce F_2 birds, and with LL and HH females from the S6 generation to produce the BC birds. The birds were vaccinated with *E. coli*, SRBC and NDV, and their immune response to each of these three antigens was individually determined.

This part of the BARD project is described in details in the attached manuscript titled: "Detection of RFLP Markers Associated with Antibody Response in Meat-Type Chickens: Haplotype/Genotype, Single-Band and Multi-Band Analyses of RFLP in the Major Histocompatibility Complex" by Yonash, Hillel, Heller, and Cahaner (under review in Journal of Heredity).

In summary, improving disease resistance in poultry by direct selection or by selecting for immune response is hardly feasible due to the quantitative nature of these traits, their low heritability and the difficulties associated with reliable measurements. In this situation, marker-assisted selection (MAS) is expected to be a more effective breeding approach. The major histocompatibility complex (MHC), known to affect immune response and disease resistance, was examined as a set of candidate genes for association between DNA markers and antibody response. Backcross (BC₁) and F₂ families were generated from a cross between lines divergently selected for high or low antibody response to *Escherichia coli* vaccination. Restriction fragment length polymorphism (RFLP) analysis of the highly polymorphic MHC class IV (B-G) region suggested an association with antibody response to several antigens (*E. coli*, SRBC, NDV). The multi-band data generated with the class IV probe were used to compare the efficacies of three alternative analyses: "single-band" (carriers vs. non-carriers of each RFLP band separately), "multi-band" (multiple regression on all RFLP bands), and "genotype" (determined from family analysis of RFLP patterns/haplotypes). Groups of birds identified by the "multi-band" analysis were identical to the haplotype-based genotypes, suggesting that the laborious step of haplotype determination can be omitted without unduly sacrificing power of analysis.

3.1.2.2 Phase 2: Genotyping a resource population of F₂ with three MHC-related probes.

Phase 2 of the search for association between antibody response and MHC-related RFLP marker consisted of 80 F₂ birds from the resource population used in Phase 1. This part of the BARD project is described in full detail in the attached page proof titled: "Major histocompatibility complex (MHC) related cDNA probes associated with antibody response in meat-type chickens" by Yonash, Kaiser, Heller, Cahaner and Lamont (Animal Genetics, in press).

In summary, the major histocompatibility complex (MHC) region was examined as a set of candidate genes for association between DNA markers and antibody response. Intercross F_2 families of chickens were generated from a cross between high (HC) and low (LC) *E. coli* antibody lines. Restriction fragment length polymorphism (RFLP) analysis was conducted by using three MHC-related cDNA probes: chicken MHC class IV (B-G), chicken MHC class I (B-F), and human MHC-linked *Tap2*. Association between RFLP bands and three antibody response traits (*Escherichia coli*, sheep red blood cells and Newcastle disease virus) were determined by statistically analyzing each band separately, and also by analyzing all bands obtained from the three probes by using multiple regression analysis. The MHC class IV probe was the highest in polymorphisms but had the lowest number of bands associated with antibody response. The MHC class I probe yielded 15 polymorphic bands of which four exhibited association with antibody response traits. The *Tap2* probe yielded 20 different RFLP bands of which five were associated with Ab production. Some *Tap2* bands were associated with multiple antibody response traits. The multi-band analysis of the three probes' bands revealed more significant effects than the analysis of each band separately. This study illustrates the efficacy of use of multiple MHC region probes as candidate markers of quantitative trait loci (QTLs) controlling antibody response in chickens.

3.1.3 Association Between Microsatellite Markers and QTL for Immune Response

3.1.3.1 Phase 1. Screening F_2 and BC_1 populations derived from HHxLL cross in the S_5 generation

The main part of the search for microsatellite markers consisted of a genome-wide screening of a population of 160 F_2 and BC_1 birds, all progeny of a single F_1 male obtained from a HHxLL cross in the S_5 generation. This population was screened by 61 microsatellite markers. Out of them, 25 markers were informative, and three were found to be significantly associated with antibody response to three antigens (*E. coli*, NDV and SRBC) or survival to a pathogenic *E. coli* challenge. These findings suggest that QTLs for immune response in broilers are located on Chromosome 2 (*Ch.2*) around position 464 cM, on Chromosome 5 (*Ch.5*) around position 174 cM, and in linkage group *E31* around position 35 cM.

This part of the BARD project is described in full details in the attached manuscript titled: "Microsatellites linked to QTL affecting antibody response and survival rate in meat-type chickens" by Yonash, Cheng, Hillel, Heller, and Cahaner (under review in Animal Genetics).

In summary, selecting for immune response parameters may lead to improved general disease resistance. Disease resistance and immune response are hard-to-measure quantitative traits with low to moderate heritability, and hence may respond more efficiently to marker-assisted selection (MAS) than to phenotypic selection. For detecting DNA markers linked to quantitative trait loci (QTL) associated with immune response, a resource half-sib family of 160 backcross (BC₁) and intercross (F₂) birds was derived from a cross between two meat-type lines divergently selected for high or low antibody (Ab) response to *E. coli*. Using 25 microsatellite DNA markers covering ~25% of the chicken genome, selective genotyping based on the *E. coli* Ab response distribution was followed by complete genotyping of the entire family with four suggestive markers. Three of these markers exhibiting significant association with immune response were identified: (1) ADL0146 on *Ch.2* associated with Ab response to sheep erythrocytes (SRBC) and Newcastle disease virus (NDV), (2) ADL0290 on linkage group *E31* affecting Ab to NDV, (3) ADL0298 on *E34* associated with Ab and survival to *E. coli*. The family was also genotyped with five linked markers from two of the suggestive regions, and the data was analyzed by a multi-marker model. The results revealed significant effects on additional Ab responses and survival rate, suggested the location of the QTL, and confirmed the correlation between immune responses and disease resistance. These findings support the idea of improving poultry immunocompetence by MAS.

3.1.3.2 Phase 2. Genotyping DNA pools from the divergently-selected lines (HH and LL).

Phase 2 was conducted in order to confirm the findings of the Phase 1, and their relevance to the genetic difference between the two divergently-selected lines, HH and LL. The specific objective of this phase was to compare the divergent lines for allele frequencies of microsatellite markers linked to the three chromosomal regions identified in Phase 1 as suggested location of QTL affecting immune response of broilers. Allele frequencies were estimated in

pooled samples of DNA. Each divergent line actually consists of independent duplicated sub-lines (H1 and H2 in line HH, L1 and L2 in line LL). DNA pools, each of 14 individuals, were prepared from each of the four sub-lines at S_{10} generation. Similar pools were prepared from 25 individuals of each sub-line at the S_{12} generation.

We attempted to genotype these eight DNA samples with ten microsatellite markers from the three chromosomal regions. Three markers were non-informative due to technical reasons, and a fourth marker was monomorphic. The six informative markers were MCW0320, ADL0146 and LEI0104 on the *Ch.2* region, ADL0166 and ADL0298 on the *Ch.5* region, and ADL0290 on the *E31* region. The exact map location of these markers, and the size of their alleles, as identified in the eight pooled DNA samples, are presented in Table 1. Except for marker ADL0166 on *Ch.5*, the other five markers were included in the Phase 1, and for each of them, the two sire alleles evaluated in Phase 1 were found also in the genotyped DNA pools, either with identical number of bp (base pairs), or with only one bp difference, which falls within the genotyping technical error.

The estimated frequencies within each pooled DNA sample was determined from the peak of each allele, either from its height relative to the height of the peaks of the other alleles of the same marker (Table 1), or from the relative area of each peak (Table 2) or better from the average of these two measurements (Table 3). The results obtained were quite similar, with no more than 5% difference between the two methods, and no consistent direction for these differences. To associate between the genetic response to the long-term selection and the genotyped markers, differences between divergent lines had to be consistent in both sub-lines of each selection direction, over the two generations.

For each chromosomal region, there was one marker with apparently a significant difference in allele frequency between the HH and the LL lines. These markers were those found in Phase 1 to be most significantly associated with immune response. The most clear and consistent differences were found for marker ADL0290 in linkage group *E31*. In the two LL sub-lines only allele 189 was present, in both generations, whereas in the HH sub-lines, the frequency of this allele averaged 32% in S_{10} , and it was completely eliminated (0%) in S_{12} (Table 3). The second allele of this marker, 174, was not found in the LL lines,

and its frequency in the HH lines averaged 68% in S_{10} and increased to 100% in S_{12} . It appears, therefore, that allele 174 is closely linked to the “increasing” allele of a linked QTL, whereas allele 189 of this marker is linked to the “decreasing” allele of this QTL.

The allele frequency of ADL0298 (on *Ch.5*) also appears to change along with the divergent selection for antibody response to *E. coli* vaccine. The frequency of allele 106 averaged 54 % in the HH lines in both generations, in contrast to an average of 13% in the HH lines in S_{10} , complete elimination of this allele from the HH lines in S_{12} . It is suggested that allele 106 is linked to an “increasing” allele of the QTL on *Ch.5*, hence it was eliminated from the LL lines, whereas none of the other two alleles of the marker (104 and 114) was exclusively linked to the “decreasing” allele of the QTL (Table 3).

On *Ch.2*, the frequencies of the alleles of marker MCW0320 apparently had changed in response to the selection. Allele 172 appears to be linked to the “decreasing” allele of the QTL in this region; it was eliminated from sub-line H2 already in S_{10} , and its frequency in H2 averaged 16% only, whereas in the LL lines, its frequencies were 38 and 58% in S_{10} , and further increased to 62 and 64% in S_{12} . Allele 176 of this marker appeared to be linked to the “increasing” allele of the QTL. Its frequency in sub-line H1 increased from 33% in S_{10} to about 50% in S_{12} , and in sub-line H2, these two additional cycles of selection increased the frequency of allele 176 from 76 to 93% (Table 3). At the same time, the frequency of this allele was reduced in sub-line L2 from about 63% in S_{10} to 34% in S_{12} , and in sub-line L1 it was only 22% in both generations.

In conclusion, the same markers that had a significant association with immune response of the F_2 and BC_1 birds in Phase 1, also differed in their frequency in the HH and LL lines. The agreement between the S_{10} and S_{12} generations, and mainly the agreement between the duplicated sub-lines, clearly suggest that these frequency differences resulted from the divergent selection. Thus, the findings of Phase 2 strongly support those of Phase 1, namely, that QTL for immune response are located in these three chromosomal regions.

3.1.3.3 Phase 3. Creating a new resource population by HHxLL cross in the S₁₁ generation.

Only about one half of the genetic variation in immune response has been explained by the detection of three putative QTL; with the increased genetic difference between the HH and LL lines, and the greater saturation of the chicken genome with microsatellite markers, it is very likely that additional QTL for immunocompetence will be detected in a new resource population derived from HHxLL cross in a more advanced generation. Learning more about the three putative QTL, and detecting microsatellite markers linked to additional QTL, will facilitate a more elaborate molecular dissection of the genetic control of immunocompetence traits and the correlation between them and with growth rate, the broilers' main performance trait.

The results of this BARD project suggest that growth rate interacts with the expression of genetic variation of immunocompetence traits, including disease resistance and overall viability and also antibody response. All the birds in the resource population used to detect marker-QTL association in this project (Phase 1) had the growth potential of mid-80's broilers (when the selection of the LL and HH lines was initiated). Therefore the expression of growth-dependent variation was limited, possible interaction of the detected QTL with growth rate could not be evaluated, and no QTL could be detected if its expression is limited to fast-growing broilers. Inclusion of genetic variation for rapid growth in a new resource population will allow the detection of QTL that affect growth-immunocompetence relationships, and thus increase the feasibility of practical application of MAS (marker-assisted selection) to improve immunocompetence and viability of young broilers. It therefore appears that laying the foundation of a new resource population should be a one of the current project's objectives.

At the S₁₁ generation of selection, HH and LL chickens were reciprocally mated to produce a new F₁ generation. Fifty F₁ females and 17 males were kept for reproduction. The new resource population will be generated by selecting several F₁ males, and mating each of them with eight from each of four genetic groups: F₁, HH, LL, and CC (fast-growing commercial females). This mating scheme will generate, within each sire family, four groups of progeny: F₂, BC to HH, BC to LL, and TS (test cross) with commercial stock. This way, for each F₁ sire, the alleles of heterozygous markers, and the alleles of QTL linked to them,

will co-segregate on four genetic backgrounds differing in growth rate and in mean and variance of immune response.

The F₁ males used as sires of the new resource population should be heterozygous to the relevant markers, especially those found to be associated to immune response (Phase 1) and to differ in allele frequencies between the selected lines (Phase 2). Therefore the 17 F₁ males were genotyped for eight microsatellite markers linked to the three putative chromosomal regions. The markers are listed in Table 4, along with the percentage of heterozygous F₁ males for each of them. Heterozygosity averaged 65.5%, ranging from 43% (ADL0290) to 100% (ADL0166). Individual heterozygosity of 16 F₁ males ranged from 50 to 87%, with an exceptional male showing only 25% heterozygosity (Table 5). Selection of F₁ males to serve as sires of the new resource population will depend on good semen production and high level of heterozygosity, mainly in the markers already found to be most significantly associated with immunocompetence.

3.2 Studies with the US Lines Under Investigation for Genetic Marker Associations with Immune Response and Disease Resistance to *Salmonella Enteritidis*

3.2.1 Additional Antibody Response System Established in Project

Three different vaccine antibody response systems (*E. coli*, *P. multocida*, and *S. enteritidis*) were examined in US commercial broiler lines to select one with a wide range of phenotypes and relatively normal distribution, as well as being reliably measured by ELISA. Six hundred broiler chicks (200 per vaccine type) were evaluated. *S. enteritidis* early vaccination was selected, based upon results of vaccine antibody from two broiler breeder lines as well as for its importance in the poultry industry, for continuing studies. Line differences and heritability (0.25 and 0.26 in two different broiler breeder male lines) detected in *S. enteritidis* antibody levels demonstrated genetic control of humoral response to this pathogen and therefore the feasibility of identifying the genetic basis of control of this response. This adds to the BARD project the study of response to another pathogen of significant concern in broiler production and food safety. In contrast to *E. coli*, which is primarily a secondary pathogen, *S. enteritidis* is a primary pathogen. The details of this investigation are given in the attached paper, titled, "Effects of genetics, vaccine dosage, and postvaccination sampling

interval on early antibody response to *Salmonella enteritidis* vaccine in broiler breeder chicks” by Kaiser, Wing, and Lamont (Poultry Science 77:271-275, 1998).

In summary, broiler breeder chicks of two different genetic lines were evaluated for early antibody response to *Salmonella enteritidis* (SE) vaccine. Antibody responses to three dosages of SE vaccine administered at 22 d of age were measured at 3, 6 and 10 d postvaccination. Within each line, antibody levels at 10 d postvaccination were significantly higher than at either 3 or 6 d postvaccination. At all vaccine dosages, there was a significant antibody-response difference between the genetic lines at 6 and 10 d postvaccination. The vaccine dosage significantly affected antibody levels in one of the two genetic lines. These results demonstrate a genetic component of early antibody response to SE vaccine in broiler breeder chicks.

3.2.2 Relationship of SE Antibody Response and Disease Resistance Confirmed

Because many components of the immune response may be involved in resistance to any specific disease, it was important to confirm a relationship between SE antibody titers and the response to live SE challenge. Reliable systems for measuring exposure of birds to live *Salmonella enteritidis* were established, along with protocols for quantification of bacterial burden, which allowed investigations of relationship between genetics of antibody production and bacterial burden of SE. Pedigreed chicks were hatched and individually wingbanded, then transferred to BL-2 biosecurity level isolation facilities. Chicks were housed in three separate rooms, providing experimental replication within each hatch. Chicks from each hatch underwent SE challenge test without vaccination (an additional three chicks were euthanized on day of hatch, necropsied, and spleen and cecal contents were cultured to confirm lack of *Salmonella*). The test chicks were inoculated by intra-esophageal intubation with the optimal dose identified in the pilot study of virulent SE Phage type 13a at day of hatch. Their condition was monitored twice daily. Necropsies were performed on half of the birds each at 6 and 7 days. Spleen and cecal content were aseptically collected for bacterial counts. Culture and quantification of SE was determined by a quantitative measure of bacterial burden in spleen and cecal lumen content. Techniques are modified from published protocols (Gast and

Beard, 1990; Manning et al., 1994). One gram of spleen and a swab of cecal content were cultured 24 h in 10 ml of selenite enrichment broth (DIFCO, Detroit, MI 48232) at 37 C. A swab of each 24 h culture was plated onto brilliant green agar (BGA) plates (100 ug/ml Nalidixic acid) to identify the presence of SE and incubated for 24 h at 37 C (the enrichment broth is kept stored at 4 C). The plates were scored for the presence or absence of SE colonies. Questionable colony identification was clarified with a *Salmonella* O antiserum group D microagglutination test (DIFCO, Detroit, MI). Enrichment cultures from SE positive plates were then serially diluted and plated, in duplicate, on BGA plates to quantify SE concentration. The genetic relationship between SE colonization and SE vaccine antibody response was determined in broiler breeder chicks. Full-sib groups of chicks were either intraesophageally inoculated with live pathogenic SE or vaccinated against SE. Antibody response to vaccination in vaccinated chicks, and the number of SE in cecal swab cultures of challenged birds, were strongly negatively correlated (-0.87) (Lamont, 1998; PS 77-1111-1118). Thus, the genetic potential for greater SE vaccine antibody response is associated with lesser SE colonization in SE exposed broiler breeder chicks. This result establishes the basis for genetic improvement of resistance to SE by selection for antibody response to SE vaccination or by molecular markers associated with antibody response.

3.2.3 Microsatellite Polymorphism Evaluated in the Experimental Populations

3.2.3.1 Phase I: Microsatellite polymorphisms in broiler populations.

The publicly available chicken microsatellite primer sets in the US Poultry Genome Coordinator Mapping Kits were mostly developed from White Leghorn or Jungle Fowl DNA sequences. Only a limited number of investigations have studied utilization of these microsatellites across diverse chicken populations other than Jungle Fowl and Leghorn. To be effectively used in diverse populations, microsatellite primers need to efficaciously amplify polymorphic DNA product. This study was designed to evaluate the utility and informativeness in broiler lines of microsatellite primers developed from Leghorn or Jungle fowl sequence.

The results of these studies are detailed in the attached manuscript by Kaiser, Yonash, Cheng, Cahaner, and Lamont, entitled "Microsatellite Polymorphism Between and Within Broiler Lines".

In summary, the two independent populations of this study were genotyped with microsatellite markers to determine genetic polymorphisms both within and among broiler populations. One was a broiler breeder male line studied for SE response. The other was derived from a commercial broiler line by divergent selection for EC antibody response, and the typed individuals represented F₁ progeny of high-antibody and low-antibody lines. Six individuals per line were genotyped with primers from the US Poultry Genome Mapping Kits 1 and 2. The 59 primer sets provided wide genomic coverage. All primers amplified a PCR product in the SE line, but two produced no product in the EC line. Only one primer pair yielded monomorphic product in both lines. Of the primers generating product, there were 91.5 and 89.5% polymorphic primer pairs, respectively, for the SE and EC lines. The average allele number per microsatellite was 2.8 and 2.9, respectively, for SE and EC lines. Considering the 57 primer pairs generating product in both lines, only 70 (27.7%) of 253 total alleles were shared between lines, with 44 primer pairs sharing alleles between lines. This study illustrates the highly polymorphic nature in broiler lines of microsatellites developed from Red Jungle Fowl or White Leghorn.

3.2.3.2 Phase II: Microsatellite profiles in highly inbred chicken lines

The role of inbred lines in biological research has fundamental importance in providing genetic diversity between lines and genetic consistency within lines. Inbred lines can serve as excellent parent lines for development of resource populations to map genes or QTL in widely divergent crosses. It is rare to have highly inbred lines available in agricultural animals, but such lines were developed and are maintained at Iowa State University. To assess the efficacy of use of these lines in producing crosses to identify markers linked to QTL for immune response and disease resistance, microsatellite marker profiles were developed for many highly inbred chicken lines.

The results of these studies are detailed in the attached page proof by Zhou and Lamont entitled, "Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers", *Anim. Genet.* (in press).

In summary, forty-two microsatellite loci were analysed in 23 highly inbred chicken lines derived from Leghorn, Jungle Fowl, Fayoumi and Spanish breeds. Line-specific alleles among breeds and lines were detected. The band-sharing (BS) values were calculated and the proportion of shared alleles distances (Dps) were estimated. The BS values and Dps between sets of MHC-congenic lines ranged from 0.74 to 0.96, and 0.05 to 0.35, respectively. The BS values between each pair of noncongenic Leghorn lines were 0.32 to 0.97, and between Leghorn and exotic (Jungle Fowl, Fayoumi and Spanish) breeds were 0.03 to 0.55. The Dps between Fayoumi lines and other lines were much larger (0.66-1.34) than within Leghorns, and the Jungle Fowl breed had the largest distances with other lines (1.12-5.38). The phylogenetic consensus tree that was constructed grouped these 23 inbred chicken lines into four different clusters. These results are in accordance with the origin and breeding history of these inbred lines, which indicates that the use of microsatellites for the study of genetic biodiversity is accurate and reliable. In addition, the significance and value of inbred chicken lines in molecular genetic research is discussed.

3.2.4 Resource Populations Developed; Phenotypic Data, DNA Collected, Assays Conducted

Table 6 presents information on the populations that have been used in the US-based portion of the live-animal studies. The 600 birds on the first 3 lines ("Pretest") were used to evaluate the 3 different vaccine systems, as described in section 3.2.1. The subsequent populations are part of a four-generation full-pedigreed population structure of pure broilers, highly inbred lines, and their F1 and BC1 populations, designed and tested to evaluate the genetics of antibody response and disease resistance to *Salmonella enteritidis*. Figure 1 shows this population structure, which contained over 3500 birds. Males and females of broiler Line 1 produced offspring in 2 hatches in September, 1996, which were either challenged with pathogenic SE (Exp) or evaluated for SE vaccine antibody response (Vac). This set of experiments determined the genetic correlation of SE vaccine antibody production potential and bacterial burden after SE challenge. Based on SE vaccine phenotype, selected broilers from the September 5, 1996, hatch were mated to produce from hatches in May to

September, 1997 that were evaluated for SE vaccine response, response to SE challenge after vaccination, or response to SE challenge alone.

A smaller subset of the male broiler breeders were selected to produce two hatches (October and November, 1997) in matings with either broiler dams or with dams from highly inbred lines. These broiler and broiler by inbred F₁ chicks were evaluated for SE vaccine antibody response. From the antibody response, microsatellite genotype, and pedigree information, birds of the October and November, 1997 hatches were mated to produce either pure broiler or BC, (by mating broiler F₁ sires with inbred dams) chicks in August, 1998, which were evaluated for SE vaccine antibody response. A comparison of the SE vaccine antibody levels of each population that was SE-vaccinated (not challenged) is given in Table 7. In comparison of pure broilers with the inbred lines, the broiler antibody response is intermediate to that of the Leghorn-derived (GHs6 and GHs13) and the "exotic" (Spanish and Fayoumi) origin lines. In general, the broiler x inbred crosses (both F₁ and BC₁ generations) suggest heterosis in antibody level.

Multiple hatches of the broiler x inbred F₁ chicks (January, March, May, 1998) and broiler x inbred BC₁ chicks (September, October, 1998) were placed into challenge studies with pathogenic SE. This extensive, unique full-pedigreed four-generation population with phenotypic data on SE vaccine antibody response, resistance to pathogenic SE and DNA or blood samples for DNA isolation available from all individuals established a powerful resource population structure to dissect the genetic control of response to *Salmonella enteritidis*.

3.2.5 *Salmonella enteritidis* Population Screening with Microsatellites

In Phase I of the process of identifying DNA markers for SE response, 79 microsatellite primers were screened, including the ones used on the *E. coli* (EC) population. Fifty-eight microsatellites were selected for further analysis, based on the criteria that they were polymorphic in a majority of the SE population's foundation sires, were easily scored, and provided wide genomic coverage. The 15% of the broiler individuals representing the antibody phenotypic extreme for high and low tails of the antibody distribution were used to make DNA pools. Allele frequency differences between extreme pools were observed as DNA band fluorescence intensity differences as measured on an ABI machine. Microsatellite

markers with alleles that differ in frequency between extreme phenotype pools are presumed to be a putative marker of antibody response QTL. Fifteen microsatellites were observed to have a very marked allele frequency difference between the phenotypic extreme pools.

The phenotypic extremes, by sire family, were then selectively genotyped with these 15 microsatellites, of which 4 were selected as markers to be further investigated in designed matings. The selected microsatellites were: ADL 0020, ADL 0138, ADL 0198 and ADL 0298. The four microsatellites were chosen for use based on the large magnitude of allele frequency differences between the half-sib extreme pools, distributed chromosomal location, association with immune response in *E. coli* lines, and presence in heterozygous state in multiple sires.

Over 50 male broiler progeny were then individually genotyped with the four microsatellites. Four males, which were heterozygous for one or more of the four microsatellites and were from the phenotypic extreme group in which the microsatellite allele was present in higher frequency, were selected as next-generation sires. These sires were mated to unrelated broiler dams from the respective opposite antibody phenotypic extreme to generate a microsatellite marker-segregating population with a large phenotypic response range, to be evaluated for marker genotype and phenotype of SE vaccine response and disease resistance. In the same generation, genotyped male broilers were mated with females of the broiler line, or with one of four highly inbred lines derived from Leghorn or exotic breed origins, to produce F₁ broiler x inbred progeny.

At each generation, hatches were produced to measure SE vaccine antibody response and to evaluate several parameters of response to challenge with pathogenic SE. Blood samples for DNA isolation and serum samples for antibody evaluation were collected from all birds in the 4-generation pedigreed study (n = 3532). The 4-microsatellite genotyping of three generations of pure broilers and the F₁ population of broiler x inbred lines is completed. The data of the pure broilers and F₁ populations are currently being analyzed for relation of genotype with SE antibody response and disease resistance. In the next generation, F₁ (broilers x inbred) males were mated with females of each relevant inbred line to generate BC₁ offspring to test for phenotype and genotype as described for the F₁ generation. Phenotypic data on SE vaccine antibody

response are now collected on the BC1 generation and simultaneous hatches of the counterpart pure broiler and pure inbred lines. The response to pathogenic SE challenge was also evaluated with two hatches of BC1 chicks. The genotyping remains to be done, as well as statistical analysis of genotype-trait associations.

4. EVALUATION OF THE RESEARCH ACHIEVEMENTS AS RELATES TO THE ORIGINAL RESEARCH PROPOSAL AND ITS OBJECTIVES

The successful completion of this BARD project resulted in the identification of several DNA markers -- both MHC-linked candidate genes and non-MHC-linked microsatellites -- that account for much of the genetic variation in antibody response to *E. coli* vaccine. These immunocompetence-QTL-containing chromosomal regions therefore control much of the variation in a quantitative immune-response trait. The project was also enhanced by the addition of a second pathogen evaluation system, *Salmonella enteritidis*. This brings a bacterial model of an important primary pathogen for both live animal production and food safety. The validity of SE vaccine antibody response as a genetic correlate of bacterial burden was established, and putative DNA markers of QTLs were identified. The identification of these chromosomal regions is the necessary prerequisite to a molecular dissection of the genetic direct effects of these regions and the QTLs contained therein, and their interactions with each other, with other traits, and with diverse genetic backgrounds. The BARD project also provided much additional useful information, genetic resources, research materials, and reliable experimental protocols and systems, as detailed in the preceding paragraphs of this report. Importantly, some DNA markers were associated not only with antibody response to *E. coli* vaccine, but also with antibody response to three other antigens including *Salmonella*, and to mortality after challenge with pathogenic *E. coli*. This illustrates the validity of the assumption that specific QTLs identified by DNA markers may have generalized effects and therefore be efficacious selection criteria in marker-assisted selection to improve general resistance to disease.

5. DESCRIPTION OF COOPERATION

The investigators in this project have a proven record of efficient cooperation throughout the last several years. This cooperation has yielded 10 joint research reports, of which 5 are published papers or manuscripts in press or in review from work

conducted during this four-year BARD project (1994-1998). The cooperation in this BARD project includes synergy, complementation and mutual support, which takes advantage of the unique expertise, experience, and resources of each investigator.

The investigators were in contact several times weekly for planning, discussion, and coordination of the BARD research. In addition to the BARD-funded trips of the PIs to the cooperators' laboratory for intensive discussions and data analysis, travel funds were leveraged with support available from other sources. This resulted in an average of one on-site visit per year, which enhanced coordination of research protocols, transfer of research materials and drafting of manuscripts. The PIs and other researchers in the BARD project also took advantage of mutual attendance at one or two of the same scientific conferences each year to conduct additional coordination activities related to the BARD project.

The high level of integration of work plans is clearly illustrated by the joint authorship of several papers. As examples of integrated work, samples of DNA or membranes containing digested DNA from birds of the Israeli *E. coli* populations were sent to Iowa for RFLP analysis with MHC-specific gene probes. Statistical analyses were conducted by the Israeli team, and the manuscripts were jointly authored. Broiler DNA from the Israeli and US lines were studied for microsatellite diversity between the two experimental lines and a manuscript was jointly authored.

6. CONCLUSIONS

1. Populations of broiler chickens can be effectively selected for vaccine response to bacterial antigen by phenotypic selection.
2. Genetic potential for antibody production to bacterial vaccines is positively correlated with resistance to disease caused by pathogenic strains of the same bacteria.
3. Molecular markers within the major histocompatibility complex region partly control antibody response to bacterial vaccines.
4. Microsatellite markers can be effectively used to screen the chicken genome to identify non-MHC markers associated with vaccine antibody response.

5. Several molecular markers are associated with multiple traits of antibody response and resistance to disease indicating the generalized effects of markers identified by association with specific traits.
6. Interaction of genetic background with antibody response is significant and highlights the need to evaluate marker effects in multiple genetic backgrounds and environments.
7. Overall results indicate the effectiveness of phenotypic selection and the potential of molecular-marker assisted selection to enhance production efficiency in meat-type birds by reducing bacterial disease susceptibility.

7. LIST OF PUBLICATIONS

- Cahaner, A., N. Deeb, R. Yunis, and Y. Lavi, 1998. Reduced stress tolerance in fast growing broilers. Proc. 10th European Poultry Conference, Jerusalem, Israel, Vol. I, pp. 113-117.
- Cahaner, A., N. Yonash, G. Leitner, and E.D. Heller, 1995. The genetics of immune response and resistance to *E. coli* of meat-type chickens. Proc. AVIAGEN, 11th Inter. Symp. Current Problems in Avian Genetics, Krakow (Poland), pp. 232-235.
- Cahaner A., N. Yonash, R. Yunis, J. Hillel, D. Heller, M. G. Kaiser, and S. J. Lamont, 1997. QTL identification in a cross between lines selected divergently for high and low immune response to *E. coli*. Proc. 3rd Eur. Poultry Breeders' Roundtable. Sept. 3-5, 1997, Prague, Czech Republic, pp. 92-105.
- Kaiser, M. G., T. Wing, A. Cahaner, and S. J. Lamont, 1997. Line difference and heritability of early antibody response to *Salmonella enteritidis* vaccine in broiler breeder chicks. Proc. AVIAGEN, 12th International Symp. Current Prob. In Avian Genet. Prague, Czech Republic, pp. 138-139.
- Kaiser, M. G., T. Wing, and S. J. Lamont, 1998. Effects of genetics, vaccine dosage, and postvaccination sampling interval on early antibody response to *Salmonella enteritidis* vaccine in broiler breeder chicks. Poultry Sci. 77:271-275.
- Kaufman, J. F. and S. J. Lamont, 1996. pp. 35-64. The chicken major histocompatibility complex. In: *The Major Histocompatibility Complex in Domestic Animal Species*. S. J. Lamont and L. B. Schook, eds., CRC Press, Boca Raton, FL.

- Lamont, S. J., 1998. Impact of genetics on disease resistance. *Poultry Sci.* 77:1111-1118.
- Lamont, S. J., 1998. The chicken major histocompatibility complex (MHC) and disease. In: *Genetic Resistance to Animal Diseases*. OIE Scientific and Technical Review, Vol. 17(1), 128-142.
- Kaiser, M. G., N. Yonash, H. H. Cheng, A. Cahaner, and S. J. Lamont, 1998. Microsatellite polymorphism among and within broiler chicken lines. pp. 94. In: *Proc. Plant and Animal Genome VI*. San Diego, CA, January 18-22.
- Pinard-van der Laan, M.-H., P. B. Siegel, and S. J. Lamont, 1998. Lessons from selection experiments on immune response in the chicken. *Poultry & Avian Biology Reviews* 9(4):125-141.
- Yonash, N., G. Leitner, R. Waiman, E.D. Heller, and A. Cahaner, 1996. Genetic differences and heritability of antibody response to *Escherichia coli* vaccination in young meat-type chicks. *Poultry Sci.* 75:683-690.
- Yonash, N., M. G. Kaiser, E. D. Heller, A. Cahaner, and S. J. Lamont, 1999. Major histocompatibility complex (MHC) related cDNA probes associated with antibody response in meat-type chickens. *Anim. Genet.* (in press).
- Yunis, R., A. Ben-David, E. Berman, E. D. Heller, and A. Cahaner, 1997. Genetic differences in immune response of broilers reared under commercial conditions. *Proc. AVIAGEN, 12th International Symp. Current Prob. In Avian Genet.* Prague, Czech Republic, pp. 173-174.
- Yunis, R., D. Heller, J. Hillel, and A. Cahaner, 1998. Searching for DNA microsatellite markers associated with antibody response in young meat-type chickens. *Proc. 10th European Poultry Conference, Jerusalem, Israel, Vol. I*, pp. 288-293.
- Zhou, H. and S. J. Lamont, 1999. Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. *Anim. Genet.* (in press).

Table 1: Relative peak height of alleles of the microsatellite markers, in DNA pools of the selected sub-lines from two generations of selection

Linkage group	Marker	Map ¹ location	Allele size (bp)	Sub-Lines of S ₁₀						Sub-Lines of S ₁₂					
				H1	H2	HH	L1	L2	LL	H1	H2	HH	L1	L2	LL
				----- Relative peak height (%) -----						----- Relative peak height (%) -----					
Ch. 2	LEI0070	378		NP ²	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	MCW0320	409	168	57.1	23.2	40.1	20.2	0.0	10.1	29.3	7.1	18.2	15.2	0.0	7.6
			172	10.2	0.0	5.1	57.6	38.4	48.0	23.2	0.0	11.6	62.6	64.7	63.6
			176	32.7	76.8	54.8	22.2	61.6	41.9	47.5	92.9	70.2	22.2	35.4	28.8
	ADL0146	464	153 164	57.6	69.7	63.6	41.4	60.6	51.0	NP	NP	NP	NP	NP	NP
E31	LEI0104	468	221	65.7	46.5	56.1	67.7	53.5	60.6	NP	72.7	72.7	78.8	19.2	49.0
			225	34.3	53.5	43.9	32.3	46.5	39.4	NP	27.3	27.3	21.2	80.8	51.0
				NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	ADL0304	7		OL ³	OL	OL	OL	OL	OL	CL	OL	OL	OL	OL	OL
	MCW0217	24		62.6	72.7	67.7	0	0	0	NP	100	100	0	0	0
Ch. 5	ADL0290	35	174	37.4	27.3	32.3	100	100	100	NP	0	0	100	100	100
			189	100	100	100	100	100	100	100	100	100	100	100	100
			227	42.7	0.0	21.1	0.0	0.0	0.0	36.1	0.0	18.0	0.0	0.0	0.0
	ADL0166	138	131	20.8	36.7	28.9	35.4	33.3	34.3	16.5	19.6	18.0	38.8	7.1	22.8
			139	14.6	27.6	21.1	0.0	0.0	0.0	22.7	24.7	23.7	28.6	17.2	22.8
145			9.4	35.7	22.7	64.7	66.7	65.7	11.3	24.7	18.0	32.7	42.4	37.6	
ADL0298	174	149	12.5	0.0	6.2	0.0	0.0	0.0	13.4	30.9	22.3	0.0	33.3	16.8	
		151	40.4	31.3	35.9	44.9	29.3	37.1	NP	19.4	19.4	42.4	47.5	45.0	
		104	39.4	68.7	54.0	26.5	0.0	13.2		54.1	54.1	0.0	0.0	0.0	
			114	20.2	0.0	10.1	28.6	70.7	49.7	26.5	26.5	57.6	52.5	55.0	

¹ Location of marker on the Esat Lansing chicken genome map

² NP = No products were obtained in the PCR reactions

³ OL = Over loaded onto the gel

Table 2: Relative peak area of the microsatellite markers in DNA pools of the selected sub-lines from two generations of selection

Linkage group	Marker	Map ¹ distance	Allele size (bp)	Sub-Lines of S ₁₀						Sub-Lines of S ₁₂					
				H1	H2	HH	L1	L2	LL	H1	H2	HH	L1	L2	LL
				----- Relative peak area (%) -----						----- Relative peak area (%) -----					
Ch. 2	LEI0070	378		NP ²	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				57.1	24.2	40.6	17.2	0.0	8.5	27.6	6.1	16.8	10.2	0.0	5.1
				9.2	0.0	4.6	57.6	34.3	46.0	18.4	0.0	9.1	67.4	66.7	67.0
				33.7	75.8	54.8	25.3	65.7	45.5	54.1	93.9	74.1	22.5	33.3	27.9
				51.5	67.7	59.6	32.3	56.6	44.4	NP	NP	NP	NP	NP	NP
E31	ADL0146	464	153 164	48.5	32.3	40.4	67.7	43.4	55.6	NP	NP	NP	NP	NP	NP
				65.7	39.4	52.5	67.7	58.6	63.1	NP	71.7	71.7	80.8	16.2	48.5
				34.3	60.6	47.5	32.3	41.4	36.9	NP	28.3	28.3	19.2	83.8	51.5
				NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				OL ³	OL	OL	OL	OL	OL	OL	OL	OL	OL	OL	OL
Ch. 5	ADL0290	35	174 189	54.6	81.8	68.2	0	0	0	NP	100	100	0	0	0
				45.5	18.2	31.8	100	100	100	NP	0	0	100	100	100
				100	100	100	100	100	100	100	100	100	100	100	100
				35.7	0.0	17.8	0.0	0.0	0.0	30.6	0.0	15.4	0.0	0.0	0.0
				21.4	37.4	29.4	24.2	17.2	20.7	17.4	19.6	18.5	29.6	7.2	18.5
Ch. 5	ADL0166	138	131 139 145 149 151	20.4	20.2	20.3	0.0	0.0	0.0	28.6	29.9	29.2	27.6	13.4	20.5
				5.1	42.4	23.9	75.8	82.8	79.3	5.1	15.5	10.3	42.9	35.1	39.0
				17.4	0.0	8.6	0.0	0.0	0.0	18.4	35.1	26.7	0.0	44.3	22.0
				32.3	31.3	31.8	37.4	18.2	27.8	NP	16.2	16.2	46.5	53.5	50.0
				42.4	68.7	55.6	22.2	0.0	11.1	NP	50.5	50.5	0.0	0.0	0.0
Ch. 5	ADL0298	174	104 106 114	25.3	0.0	12.6	40.4	81.8	61.1	33.3	33.3	33.3	53.5	46.5	50.0

¹ Location of marker on the Esat Lansing chicken genome map

² NP = No products were obtained in the PCR reactions

³ OL = Over loaded onto the gel

Table 3: Allele frequencies of the microsatellite markers in DNA pools of the selected sub-lines from two generations of selection, estimated by averaging Relative peak height and Relative peak area for each allele

Linkage group	Marker	Map ¹ distance	Allele size (bp)	Sub-Lines of S ₁₀						Sub-Lines of S ₁₂						
				H1	H2	HH	L1	L2	LL	H1	H2	HH	L1	L2	LL	
				----- Relative peak area (%) -----						----- Relative peak are (%) -----						
Ch. 2	LEI0070	378		NP ²	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	
	MCW0320	409	168	57.1	24.2	40.6	17.2	0.0	8.5	27.6	6.1	16.8	10.2	0.0	5.1	
			172	9.2	0.0	4.6	57.6	34.3	46.0	18.4	0.0	9.1	67.4	66.7	67.0	
			176	33.7	75.8	54.8	25.3	65.7	45.5	54.1	93.9	74.1	22.5	33.3	27.9	
	ADL0146	464	153 164	51.5	67.7	59.6	32.3	56.6	44.4	NP	NP	NP	NP	NP	NP	
E31	LEI0104	468	221 225	65.7	39.4	52.5	67.7	58.6	63.1	NP	71.7	71.7	80.8	16.2	48.5	
	ADL0304	7	225	34.3	60.6	47.5	32.3	41.4	36.9	NP	28.3	28.3	19.2	83.8	51.5	
			MCW0217	24		NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
			ADL0290	35	174 189	54.6	81.8	68.2	0	0	0	NP	100	100	0	0
	MCW0219	47	227	45.5	18.2	31.8	100	100	100	NP	0	0	100	100	100	
Ch. 5	ADL0166	138	131 139 145 149 151	35.7	0.0	17.8	0.0	0.0	0.0	30.6	0.0	15.4	0.0	0.0	0.0	
	ADL0298	174	139	21.4	37.4	29.4	24.2	17.2	20.7	17.4	19.6	18.5	29.6	7.2	18.5	
			145	20.4	20.2	20.3	0.0	0.0	0.0	28.6	29.9	29.2	27.6	13.4	20.5	
			149	5.1	42.4	23.9	75.8	82.8	79.3	5.1	15.5	10.3	42.9	35.1	39.0	
	ADL0298	174	104 106 114	17.4	0.0	8.6	0.0	0.0	0.0	8.4	35.1	26.7	0.0	44.3	22.0	
Ch. 5	ADL0298	174	104	32.3	31.3	31.8	37.4	18.2	27.8	NP	16.2	16.2	46.5	53.5	50.0	
			106	42.4	68.7	55.6	22.2	0.0	11.1	50.5	50.5	0.0	0.0	0.0	0.0	
			114	25.3	0.0	12.6	40.4	81.8	61.1	33.3	33.3	53.5	46.5	50.0	50.0	

¹ Location of marker on the Esat Lansing chicken genome map

² NP = No products were obtained in the PCR reactions

³ OL = Over loaded onto the gel

Table 4: Heterozygosity rate (%) among 17 F₁ males genotyped for eight microsatellite markers from three linkage groups suspected to contain QTL for antibody response

Linkage group	Map location (cM)	Marker	Heterozygosity	Difference in allele frequency between HH and LL lines (Tables 1 and 2)
Ch. 2	378	LEI0070	56%	(not tested)
	409	MCW0320	87%	YES
	464	ADL0146	69%	No
	468	LEI0104	53%	No
E31	24	MCW0217	76%	(not tested)
	35	ADL0290	43%	YES
Ch. 5	138	ADL0166	100%	Yes??
	174	ADL0298	40%	Yes?

Table 5: Heterozygosity of F1 (HHxLL) males genotyped for eight microsatellite markers from three linkage groups suspected to contain QTL for antibody response

F1 sire	Heterozygosity	Heterozygote	Homozygote	NP ¹
1602	87%	7	1	
1786	87%	7	1	
1348	75%	6	2	
1655	75%	6	2	
2112	75%	6	2	
2233	75%	6	2	
1030	62%	5	3	
1090	62%	5	3	
1123	62%	5	1	2
1377	62%	5	2	1
1746	62%	5	3	
1168	50%	4	4	
1657	50%	4	4	
1845	50%	4	4	
2018	50%	4	4	
2145	50%	4	4	
1012	25%	2	1	5

¹ NP = no PCR product was obtained

Table 6. Summary of Populations of US chicken lines evaluated for microsatellite genotype and Salmonella enteritidis phenotype.										
Population	Hatch Date	Pop. No.	Sire Selection	Dam Selection	SE Vac	Exposure	ELISA	Genotype	Abbreviations	
E. coli Pretest-Broiler	3/3/95	200	RS	RS	No	No	ELISA	Blood		
PM Pretest-Broiler	2/15/96	200	RS	RS	No	No	ELISA	None	RS=Random Selection	
SE Pretest-Broiler	2/15/96	200	RS	RS	Yes	No	ELISA	None	PS=Pedigree Selection	
Commercial Broiler SE Exp	8/28/96	65	RS	RS	No	Yes	ELISA	None	MS=Marker Selection	
Broiler Line 1 SE Vac	9/5/96	329	PS	PS	Yes	No	ELISA	FCR	ELISA=antibody level assayed	
Broiler Line 1 SE Exp	9/26/96	144	PS	PS	No	Yes	ELISA	DNA	Serum=samples stored	
Broiler Line 2 SE Vac	3/13/97	331	PS	PS	Yes	No	ELISA	PCR	Blood=samples stored	
Broiler Line 1 Ver SE Vac 1	5/1/97	179	MS	PS	Yes	No	ELISA	PCR	DNA=DNA isolated	
Broiler Line 1 Ver SE Vac 2	6/5/97	180	MS	PS	Yes	No	ELISA	PCR	PCR=genotype completed	
Broiler Line 1 Ver SE VacExp	7/22/97	208	MS	PS	Yes	Yes	Serum	DNA		
Broiler Line 1 Ver SE Exp	9/16/97	200	MS	PS	No	Yes	Serum	Blood		
Broiler-pure SE Vac1	10/14/97	48	MS	PS	Yes	No	ELISA	PCR		
Broiler-F1cross SE Vac1	10/14/97	233	MS	PS	Yes	No	ELISA	PCR		
Broiler-pure SE Vac2	11/4/97	36	MS	PS	Yes	No	ELISA	PCR		
Broiler-F1cross SE Vac2	11/4/97	359	MS	PS	Yes	No	ELISA	PCR		
Broiler-F1cross SE Exp1	1/27/98	188	MS	PS	No	Yes	Serum	Blood		
Broiler-F1cross SE Exp2	3/24/98	80	MS	PS	No	Yes	Serum	Blood		
Broiler-F1cross SE Exp3	5/26/98	67	MS	PS	No	Yes	Serum	Blood		
Broiler-BC1 SE Vac 1	8/11/98	263	MS	PS	Yes	No	ELISA	Blood		
Broiler-pure SE Vac 1	8/11/98	51	MS	MS	Yes	No	ELISA	Blood		
Inbred-pure SE Vac 1	8/11/98	75	PS	PS	Yes	No	ELISA	Blood		
Broiler-BC1 SE Vac 2	8/25/98	218	MS	PS	Yes	No	ELISA	Blood		
Broiler-pure SE Vac 2	8/25/98	43	MS	MS	Yes	No	ELISA	Blood		
Inbred-pure SE Vac 2	8/25/98	60	PS	PS	Yes	No	ELISA	Blood		
Broiler-BC1 SE Exp1	9/15/98	120	MS	MS	No	Yes	Serum	Blood		
Broiler-BC1 SE Exp2	10/6/98	120	MS	MS	No	Yes	Serum	Blood		

Table 7. Antibody response to *Salmonella enteritidis* vaccination in broiler and broiler cross populations (mean $\pm$ SEM)

Population	Year	N	ELISA Antibody Value ¹
Broiler	1997	70	0.886 $\pm$ 0.014
GHs 6	1997	21	0.838 $\pm$ 0.027
GHs 13	1997	23	0.763 $\pm$ 0.036
M 15.2	1997	18	0.925 $\pm$ 0.024
Sp 21.1	1997	9	0.935 $\pm$ 0.037
F1-GHs 6	1997	162	0.848 $\pm$ 0.011
F1-GHs 13	1997	86	0.755 $\pm$ 0.017
F1-M 15.2	1997	109	0.860 $\pm$ 0.015
F1-SP 21.1	1997	57	0.796 $\pm$ 0.019
Broiler	1998	78	0.900 $\pm$ 0.014
GHs 6	1998	38	0.890 $\pm$ 0.016
GHs 13	1998	26	0.729 $\pm$ 0.026
M 15.2	1998	45	0.922 $\pm$ 0.022
BC-GHs 6	1998	132	0.830 $\pm$ 0.013
BC1-GHs 13	1998	105	0.784 $\pm$ 0.014
BC1-M 15.2	1998	135	0.886 $\pm$ 0.013
BC1-Sp 21.1	1998	51	0.893 $\pm$ 0.016

¹ELISA values of sample/positive control. In this competitive assay, higher values mean lower absolute antibody levels.

Figure 1. Schematic of major breeding populations and phenotypic test groups in the US broiler population studied for response to *Salmonella enteritidis*. Males and females of broiler Line 1 produced offspring in 2 hatches in September, 1996, which were either challenged with pathogenic SE (Exp) or evaluated for SE vaccine antibody response (Vac). This set of experiments determined the genetic correlation of SE vaccine antibody production potential and bacterial burden after SE challenge. Based on SE vaccine phenotype, selected broilers from the September 5, 1996, hatch were mated to produce from hatches in May to September, 1997, that were evaluated for SE vaccine response, response to SE challenge after vaccination, or response to SE challenge alone. A smaller subset of the male broiler breeders were selected to produce two hatches (October and November, 1997) in matings with either broiler dams or with dams from highly inbred lines. These broiler and broiler by inbred F₁ chicks were evaluated for SE vaccine antibody response. From the antibody response, microsatellite genotype, and pedigree information, birds of the October and November, 1997 hatches were mated to produce either pure broiler or BC₁ (by mating broiler F₁ sires with inbred dams) chicks in August, 1998, which were evaluated for SE vaccine antibody response. Multiple hatches of the broiler x inbred F₁ chicks (January, March, May, 1998) and broiler x inbred BC₁ chicks (September, October, 1998) were placed into challenge studies with pathogenic SE.

Figure 1

8. APPENDIX

- 8.1 Yonash, N., G. Leitner, R. Waiman, E. D. Heller, and A. Cahaner. 1996. Genetic differences and heritability of antibody response to *Escherichia coli* vaccination in young meat-type chicks. *Poultry Sci.* 75:683-690.
- 8.2 Yunis, R., A. Ben-David, E. D. Heller, and A. Cahaner. 1999. Immunocompetence and viability under commercial conditions of broiler groups differing in growth rate and in antibody response to *E. coli* vaccine. *Poultry Science* (to be submitted).
- 8.3 Yonash, N., E. D. Heller, J. Hillel, and A. Cahaner. 1999. Detection of RFLP markers associated with antibody response in meat-type chickens. *Journal of Heredity* (in review).
- 8.4 Yonash, N., M. G. Kaiser, E. D. Heller, A. Cahaner, and S. J. Lamont. 1999. Major histocompatibility complex (MHC) related cDNA probes associated with antibody response in meat-type chickens. *Animal Genetics* (in press).
- 8.5 Yonash, N., H. H. Cheng, J. Hillel, E. D. Heller, and A. Cahaner. 1999. Microsatellites linked to QTL affecting antibody response and survival rate in meat-type chickens. *Animal Genetics* (in review).
- 8.6 Kaiser, M. G., T. Wing, and S. J. Lamont. 1998. Effects of genetics, vaccine dosage, and postvaccination sampling interval on early antibody response to *Salmonella enteritidis* vaccine in broiler breeder chicks. *Poultry Science* 77:271-275.
- 8.7 Zhou, H. and S. J. Lamont. 1999. Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. *Anim. Genet.* (in press).
- 8.8 Kaiser, M. G., N. Yonash, H. H. Cheng, A. Cahaner, and S. J. Lamont. 1999. Microsatellite polymorphism between and within broiler lines. *Poultry Science* (to be submitted).

TABLE 1. Comparison of polymorphisms of 59 microsatellites in two independent broiler lines.

Polymorphism Statistics	Population		
	Line L	Line C	Lines L and C
Polymorphic primers	(54/59) 91.5 ¹	(53/59) 89.8	(58/59) 98.3
Mean number of alleles/primer	2.8	2.9	4.4
Total number of alleles	165	163	253

¹ (number of polymorphic primers/total number of primers) percent