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Identification of SNP Markers for Resistance to *Salmonella* and IBDV in Indigenous Ethiopian Chickens

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ABSTRACT: Serological data for *Salmonella* and Infectious Bursal Disease Virus (IBDV) were recorded for 760 indigenous Ethiopian village chickens raised in two distinct geographical regions, Horro and Jarso. Chickens were genotyped with a 620K SNP array. A multidimensional scaling analysis showed that the two populations were genetically distinct. In Horro chickens, genome-wide scans revealed nine SNP with chromosome-wide significant association with *Salmonella* resistance and seven SNP with genome-wide significant association with IBDV resistance. In Jarso chickens, these scans revealed one SNP with genome-wide and two SNP with chromosome-wide significant association with *Salmonella* resistance, and one SNP with genome-wide and three SNP with chromosome-wide significant association with IBDV resistance. All significant SNP for each region for either disease were located on different chromosomes. Most of these SNP had a significant additive effect and were located close to annotated genes that are known to impact the immune response in chickens.

Keywords: Ethiopia; Indigenous chickens; *Salmonella* resistance; IBDV resistance; Genome-wide association studies

Introduction

Poultry play an important role in the agriculture of many developing countries in Africa (Hassan et al. (2004)). In this regard, indigenous chickens tend to be well adapted to their local environment since they are excellent foragers, are better able to avoid predator attacks and demonstrate stronger immunity to common diseases compared to imported chickens. However, infectious diseases have a major impact on productivity, since there is very limited use of vaccination and other prophylactic measures. Genetic selection for improved disease resistance is an under-exploited, cost-effective and permanent method to control infectious diseases in poultry.

Salmonella, a zoonotic disease caused by a gram-negative enteric bacterium, and Infectious Bursal Disease (IBDV), a highly contagious immunosuppressive viral infection, have been identified as two of the most important infectious diseases in Ethiopian indigenous village chickens. Several candidate genes and quantitative trait loci (QTL) have been identified for *Salmonella* resistance in different chicken lines (Calenge et al. (2010)) but not for IBDV resistance. The objective of this study was to identify SNP markers for increased resistance to *Salmonella* and

IBDV infection in two important Ethiopian indigenous chicken populations.

Materials and Methods

Populations. The two populations of indigenous Ethiopian village chickens that were used in this study are located in Horro and Jarso, two geographical regions about 800 km from each other. The two regions are very different ecologically, economically and socially, and no poultry trade takes place between them. Four villages were randomly selected from each region for sampling; 50 farms were selected from each village and 2 chickens from each farm.

Data. In total, blood samples from 760 birds, 384 from Horro and 376 from Jarso, were collected in four rounds of field sampling over two years. Information on village, farm, shed, month of sampling, sex, age, weight and body condition score were recorded (Desta et al. (2013)). Serological data was based on single tests of individuals' sera using an in-house ELISA for *Salmonella* and a commercially available ELISA kit for IBDV. The ELISA plates used for the analysis were also recorded. DNA was extracted from the blood samples and was successfully genotyped using a high density SNP array (620K, Affymetrix).

Statistical analyses. Initially, a multidimensional scaling analysis (MDS) was performed using an IBS distance matrix to identify if there were any genetic differences between the two populations using GenABEL software (Aulchenko et al. (2007)). Samples from Horro were then analysed separately to samples from Jarso. ELISA results were reported as serum to positive ratios; for both populations and both diseases these results were log-transformed in order to obtain a normal distribution. Individual chicken records were then adjusted for the fixed effects of village, month of sampling, sex, age, weight, body condition score and ELISA plate, and the random effect of farm. Data were pre-corrected for fixed effects and the residuals used as phenotypes in a genome-wide association study (GWAS). After quality control of the Horro and Jarso data (the criteria to retain markers were: call rates 0.90, individuals with missing genotypes 0.10, Hardy-Weinberg equilibrium $P < 10^{-6}$, minor allele frequency 0.05% and 0.025% for *Salmonella* and IBDV resistance, respectively). Just over 400,000 SNP remained for further analyses. The software GEMMA (Zhou (2014)) was used to run the GWAS analyses based on a mixed model that included the

genomic relationship matrix among individuals as a random effect. After the Bonferroni correction, the $P < 0.05$ genome-wide significance threshold was set at 1.27×10^{-7} and the suggestive (i.e. one false positive per genome scan) significance threshold at 2.50×10^{-6} . In addition, a search for SNP significant at chromosome-wide level was performed. Again, the significance threshold was set at $P < 0.05$ and a Bonferroni correction for multiple testing was performed. Individual SNP found significant at genome-wide and chromosome-wide level in GWAS were further tested using a mixed model analysis to verify their significance and assess the magnitude of their effect. Furthermore, using Ensembl, the significant SNP were mapped on the reference genome (Galgal4) and a search for annotated genes within 0.5 MB windows around the significant SNP was performed.

Results and Discussion

Salmonella resistance in Horro and Jarso. The descriptive statistics of *Salmonella* serological data in the Horro and Jarso populations are given in Table 1. A higher average antibody titre was measured in Horro compared to Jarso. In addition, the variability of the data measured in Horro was higher (twice the SD) compared to that from Jarso. In Horro, GWAS revealed nine SNP located on chromosomes 3, 9, 12, 16 and 23 with a suggestive significant association with *Salmonella* resistance (Figure 1), while in Jarso one SNP with genome-wide significance was located on chromosome 17 and two SNP with suggestive significance were located on chromosomes 1 and 5 (Figure 1). All the SNP with suggestive significance at genome-wide level were also chromosome-wide significant. These SNP were still significant ($P < 0.05$) when re-analysed using the mixed linear model. All re-analysed SNP had a significant ($P < 0.05$) positive additive effect and no dominance except for a SNP on chromosome 3 of birds from Horro. The variance explained by all significant SNP collectively was 29% for Horro and 13% for Jarso. In the reference genome, many annotated genes are located within 0.5 MB of the interrogated SNP in either population. Among these genes there are some promising candidates directly related to innate immunity and hormones which might also play an important role in the immune response.

Table 1: Descriptive statistics for serological data for *Salmonella in Horro and Jarso chickens.**

Region	Mean	SD	Median	Min	Max
Horro	1.43	1.83	0.80	-0.1	19.75
Jarso	1.19	0.97	0.96	-0.2	8.37

**Salmonella* ELISA results calculated as serum to positive ratios.

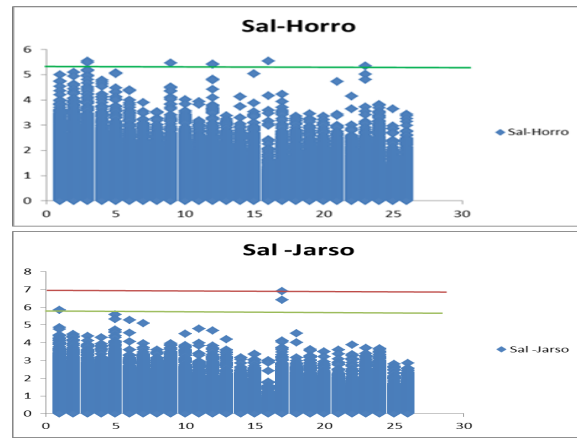


Figure 1: Manhattan plots for *Salmonella* resistance in Horro and Jarso; x-axis is chromosome number; y-axis is $-\log_{10}$ (P-value); red and green horizontal lines show the genome-wide and chromosome-wide significance thresholds, respectively.

Our results revealed different loci associated with *Salmonella* resistance in the two populations, consistent with the MDS results which showed two distinct populations. Genes controlling resistance to *Salmonella* differ depending on the chicken line used in the studies (Calenge et al. (2010)). However, two SNP identified on chromosome 16 in the Horro population are located within the genomic region carrying genes of the Major Histocompatibility Complex (MHC), which can be important for disease resistance (Zhou and Lamont (2003)). Moreover, loci on chromosome 17, close to the marker found in Jarso chickens, have also been identified to be involved in *Salmonella* resistance (Hasenstein et al. (2006)). Also, in the same region as the SNP identified on chromosome 12 in the Horro population, there is a QTL that has been linked to *Salmonella* and *Campylobacter* resistance in inbred chicken lines (Fife et al. (2011)).

IBDV resistance in Horro and Jarso. A high average antibody titre was measured in the sera of Horro chickens compared to Jarso chickens, with higher variability of the Horro data also (Table 2). In Horro, the GWAS revealed seven SNP located on chromosomes 3, 5, 7, 10 and 12 with genome-wide significant association with IBDV resistance (Figure 2), while in Jarso, one SNP located on chromosome 3 had genome-wide and three SNP located on chromosomes 4, 17 and 20 had suggestive significant association with IBDV resistance (Figure 2). The SNP with suggestive significance at genome level were also chromosome-wide significant. The significance of all the SNP was confirmed with a mixed model analysis. All SNP had a significant positive additive effect and no significant dominance effect, with the exception of the SNP located on chromosome 5 in Horro chickens which had a significant dominance effect and no significant additive effect. The variance explained by all significant SNP collectively was 29% for Horro chickens but only 1.6% for Jarso chickens, suggesting that the trait is more polygenic in the latter or there is less disease challenge there. Many annotated genes

involved in the immune response are located within 0.5 MB of these SNP. The putative QTL identified for IBDV resistance on chromosome 12 in Horro chickens is located in the same area as the SNP associated with *Salmonella* resistance in the same population. This region also harbours the QTL for *Salmonella* and *Campylobacter* resistance mentioned previously (Fife et al. (2011)). There are several candidate genes under this QTL peak, including caveolin, oxytocin receptor and interleukin-1 receptor-associated kinase-like 2.

Table 2: Descriptive statistics for serological data for IBDV* in Horro and Jarso chickens.

Region	Mean	SD	Median	Min	Max
Horro	0.063	0.175	0.0274	-0.1	1.48
Jarso	0.0089	0.123	0.0004	-0.2	0.83

*IBDV ELISA results calculated as serum to positive ratios.**Error! Not a valid link.**

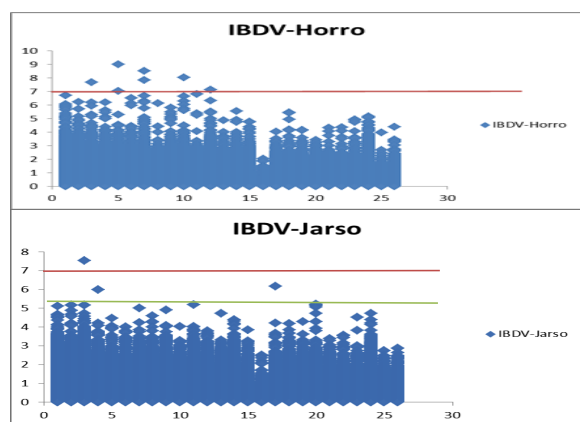


Figure 2: Manhattan plots for IBDV resistance in Horro and Jarso; x-axis is chromosome number; y-axis is $-\log_{10}(P\text{-value})$; red and green horizontal lines show the genome-wide and chromosome-wide significance thresholds respectively.

Conclusion

Almost all SNP markers for *Salmonella* and IBDV resistance identified in the two Ethiopian chicken populations had a significant additive effect and were located close to candidate genes involved in the immune response. These results are quite encouraging for the possibility of breeding for *Salmonella* and IBDV resistance in these indigenous Ethiopian chickens.

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