Immunogenetics applied to control salmonellosis in chicken: a review

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ABSTRACT

Salmonella infection is an important risk to public health, as newly hatched chicks are very sensitive to Salmonella and the infection transfers to humans through contaminated meat and eggs. Vaccination and antibiotic treatment are common strategies to control salmonellosis. However, it is doubtful whether these strategies succeed, because immune functions depend on the activity of several genes. Genetic improvement of the immune system is an effective method to control salmonellosis. There is genetic variation among breeds and individuals inside breeds in terms of resistance to infection. Genes responsible for resistance to disease can be identified through quantitative trait loci and gene expression analysis. High-throughput technologies assist us to detect single nucleotide polymorphisms (SNPs) and to analyse the regulation of several genes simultaneously. By now, several SNPs have been detected in the chicken genome that influence the function of immune genes. Additionally, the expression level of genes varies during infection depending on the breed and age of chicken and environmental conditions. This review summarizes the results of previous studies on the identification of genes and gene regulation during Salmonella infection in chickens.

1. Introduction

Salmonella enterica serovar Enteritidis and Typhimurium are the main causes of food poisoning worldwide. Chickens may be infected with Salmonella at any age, although the clinical signs may not be severe. Therefore, the infection can easily transfer to humans. The level of bacterial pathogenesis depends on both the serovar of bacteria and the immunological status of the host. Salmonella typhimurium (ST) and Salmonella enteritidis (SE) engender acute systemic disease in young chicks (Friedman et al. 2003). The host immune system, having a genetic basis, determines the level of bacterial pathogenesis. Several strategies have been introduced to prevent salmonellosis. Vaccination, sanitation and application of antibiotics are the common methods to protect domestic animals against Salmonella infections. Since vaccination against salmonellosis is not always effective, the poultry industry prefers to use antibiotics (Wigley et al. 2002). The major problems associated with the widespread use of antibiotics are the development of bacteria resistant to antibiotics, and the accumulation of antibiotic residues in food for human consumption (Baylis & Goldmann 2004). The incidence of Salmonella enterica in raw chicken meat has been documented in previous studies (Amin & Abd El-Rahman 2015; Soguilon-Del Rosario & Rivera 2015). Multidrug-resistant Salmonella enterica was detected in chicken meat samples in Egypt. Eighty-two percent of Salmonella isolated showed resistance to antibiotics (Abdel-Maksoud et al. 2015).

Another way of controlling salmonellosis is the selection of genetically resistant animals. Generally, genetic improvement of quantitative traits in livestock can be achieved by traditional selection methods based on phenotypic data or breeding values. In recent years, advances in molecular technology have created a new horizon for the genetic improvement of quantitative traits, particularly disease-resistant traits. The identification of direct or indirect molecular markers for these traits would facilitate the use of marker-assisted selection or gene introgression (Wakchaure et al. 2015).

The immune system of chickens, like in other vertebrates, includes both innate and adaptive immunity. Innate immunity is the first barrier against any infection. However, adaptive immunity includes responses correlated to lymphocytes that are active during and after exposure to an antigen. The adaptive immune system eliminates the pathogens in two ways: one through the production of immunoglobulins by B-cells, referred to as humoral immune response, and the other through cellular immune response performed by T-cells (Kean et al. 1994; Cheema et al. 2003). There are many different T-cells with their own special characteristics. For example, CD8\textsuperscript{+} are cytotoxic T-cells that kill infected cells, but CD4\textsuperscript{+} activates macrophages and B-cells as helper T-cells.

Several candidate genes have been identified that modulate the immune functions of both immune systems. The genetic map of these candidate genes, existence of single nucleotide polymorphisms (SNPs), and association of candidate gene polymorphisms with resistant traits of commercial and indigenous chickens have been assessed in several studies. The aim of this article is to review the results of the studies that have been previously done on the identification of genes involved in resistance to salmonellosis in chicken.
2. The immunobiology of avian salmonellosis

Infection caused by *S. enterica* varies depending on the type of serovar and the host genetic background. Because *S. typhimurium* and SE are major causes of salmonellosis in humans through the consumption of infected meat and eggs, understanding the mechanism of enteric or egg infection has been considered in most studies. Both SE and *S. typhimurium* can infect a range of hosts. They can persist in the gastrointestinal tract (GIT), especially in the caecum of chickens, for several months without any visible clinical signs except in early hatched chicks. Systemic disease with these serovars is more ephemeral (Barrow 2000). In contrast, *S. gallinarum* and *S. pullorum* (SP) cause systemic disease primarily in chickens and turkeys, respectively (Shivaprasad 2000). Intestinal ion permeability of chickens decreases acutely by the presence of SE. This type of response could counteract ion and fluid secretion and may explain why chickens do not develop overt diarrhoea after *Salmonella* infection (Awad et al. 2012).

Non-typhoid *Salmonella* can be detected in the intestine 6 h after inoculation. On day 12 post-infection (p.i.), *Salmonella* proliferate highly and at this time heterophils influx to the gut, indicating the key role of these cells in preventing a systemic disease in a host (Withanage et al. 2004; Cheeseman et al. 2007). Inflammation is a result of macrophages and polymorphonuclear cells. Immune cells are observed after the expression of cytokines and chemokines in the infected tissues.

The capability of invasion varies among non-typhoid *Salmonella* species. All *Salmonella enterica* are able to infect lamina propria and epithelial cells. Serovar Enteritidis is highly invasive to lamina propria, whereas serovars Typhimurium and Hadar are moderately invasive. Global gene expression of the invasive *Salmonella* serovars SE and *S. typhimurium*, and the less-invasive *Salmonella infantis* and *Salmonella hadar* was studied during infection of a chicken macrophage cell line. Major functional gene groups for intracellular physiological changes were regulated similarly in all four serovars. However, *Salmonella* pathogenicity island 1 (SPI-1) and SPI-4 genes of SE and *S. typhimurium* were strongly repressed in the macrophages, whereas *S. infantis*, *S. hadar* and other similar serovars maintained up-regulation of these gene sets. This phenomenon may explain some of the biological differences between invasive and non-invasive *Salmonella* serovars (Imre et al. 2013).

It was indicated that *Salmonella kentucky* persisted longer in chicken intestine than did *S. typhimurium*. It could be due to a higher expression of *rpos*-regulated genes in *S. kentucky* than in *S. typhimurium* (Cheng et al. 2015). The four genes *inVA*, *sopE*, *V1* and *V3* may be the virulence genes for *Salmonella*. The two latter are also called fimbria genes and they interfere in the colonization of the intestine and systemic virulence (Wagner & Hensel, 2011; Nwiji et al. 2015). During 4 days p.i. with SE, a phenotypic change occurred in the chicken caecum that induced immune signalling pathways to aid *Salmonella* to persist in the caecum for a long time. There is a chain of reactions including dephosphorylation of phospholipase C-γ1 that inhibits NF-κB signalling and activates the nuclear factor of activated T-cells signalling and blockage of interferon-γ production through the disruption of the JAK-STAT signalling pathways that mediates the induction of a host tolerance response (Kogut et al. 2016).

**Figure 1** summarizes the interaction between *Salmonella* and chicken immune system. Immune responses to *Salmonella* infection are different based on the invasiveness of the bacteria (Berndt et al. 2007). Chicken intestine has specialized epithelial cells that initiate innate immunity against infection by producing enzymes and releasing chemokines and cytokines to attract macrophages, granulocytes and immature dendritic cells (Van Immerseel et al. 2002; Wigley 2014). When innate immune cells cannot completely eliminate *Salmonella*, the secondary immune responses including cell-mediated and humoral immunity are initiated (Beal et al. 2006). *Salmonella* are picked up by macrophages and dendritic cells following the invasion of the GIT and then the spleen and liver through the lymphatic system (Wigley 2014). The interaction between *Salmonella* and macrophages has an important role in the progress of systemic diseases in both mammals and birds (Barrow et al. 1994). Macrophages generally destroy the pathogens, though *Salmonella* can adapt to this phagocytic property of macrophages particularly by the SPI-2 type III secretion system (Hensel 2000). The SPI-2 system secretes some factors that enter the host cell via phagocytic vacuoles of the macrophages. These effectors interrupt the activity of lysosomes by preventing the fusion of phagosomes, cytokine secretion and major histocompatibility complex (MHC) (Cheminay et al. 2005). While chicken intestines are the most probable site of contamination, abundant *Salmonella* have been detected on the skin of the broilers. Flagellar biosynthesis genes of *S. kentucky* including *flg A*, *flg C*, *flg K*, *flg B* and *flg J* are responsible for the surface attachment. Mutation in these genes can attenuate the ability of bacteria to attach to poultry skin (Salehi et al. 2016).

3. Immunogenetics

Developed disease control measures include good sanitation, vaccination and host genetics. Attention to the latter is important since it is permanent and free of some problems such as microbial resistance to antibiotics and low effectiveness of vaccines (Lamont et al. 2008). The first attempts for the genetic improvement of disease resistance are traced back to the 1930s when different chicken lines or breeds were examined for disease resistances or susceptibilities (Calenge et al. 2010). The second step was the estimation of heritability of disease resistance to confirm that the observed variation in resistant traits had a genetic basis (Berthelot et al. 1998).

High-throughput technologies such as microarray analysis and RNA sequencing (RNA-seq) are able to detect a large number of genes as well as their relationship. RNA-seq analysis will help to understand host–pathogen interaction and elucidate the mechanism of host genetic control of disease, and provide the basis for future studies that can lead to the development of marker-based selection of highly disease-resistant chickens (Truong et al. 2015). Both microarray and real-time PCR revealed 19 genes up-regulated in chicken embryo fibroblasts (CEF) 1 h after infection with SE. These genes were classified into three functional clusters: response to pathogens...
and other stimuli, regulation of defense activity, and cellular interaction and signalling (Szmolka et al. 2015). Thirteen genes were also induced in the caecum of orally infected chickens 4 days post-infection. A strong co-expression of interleukins (ILs) including IL1β, IL8L1 and IL6 was identified in CEF (Szmolka et al. 2015). Pathway analysis and gene ontology (GO) have produced large amounts of information about gene expression and biological pathways related to salmonellosis in chicken. However, the function of some identified genes is not clear yet. GO revealed that haemopoiesis, lymphoid organ development, immune system development and cell activation were highly active in the spleen of the group infected by S. pullorum. The systems related to innate immunity such as response to bacteria, defence response and blood circulation were highly active in the caecum of SP infected chickens (Ma et al. 2014).

The estimated heritability for different immune traits in chicken ranges from low to moderate, showing that some traits such as antibody level with heritability around 0.2–0.3 can be improved by selection. The estimated heritability for resistance to salmonellosis ranges from 0.06 to 0.26 (Berthelot et al. 1998; Kaiser et al. 1998). This wide range shows that resistance to Salmonella may be improved by genetic selection. In contrast, cell-mediated or phagocytic responses have low selection response because of low heritability (0.05–0.15). Although selection based on one immune trait may improve resistance to different diseases but not all, a special disease must have its own selection programme (Cheng et al. 1991; Lamont et al. 2003). A selection programme for the improvement of the immune system should not include a few important traits, as there is a negative correlation between immune traits and economical traits (Lamont et al. 2003). On the other hand, the interaction between genetic and environmental effects should be considered. Selection response for the improvement of the immune system in high-hygiene environments such as in breeding companies may differ from that in low hygiene environments such as commercial flocks. In this kind of condition, protection against different types of pathogens becomes a main objective of selection programmes; another point is the nutrient requirements of improved immune system and genetic correlation between immunity and growth traits, which remain to be more clear.

In recent years, many studies have been performed to detect the gene structure of Salmonella and its ability to resist against antibiotics. Salmonella invasion protein C gene (Sip C), a gene that intercedes in the rearrangement of the actin cytoskeleton of the bacteria, can be cloned to pGEM T-easy vector. This gene construction is useful for engineering attenuated vaccine against S. typhimurium (Safarpour Dehkordi et al. 2015).

4. Genetic variation

Identification of molecular markers associated with disease resistance is one of the sustainable approaches to improve immune traits (Lamont 1998). Some immune genes have a positive correlation with growth traits possibly because better health causes better growth (Ye et al. 2006). In contrast, some genes with their main activity in immunity have a negative correlation with
growth. For example, animals with high levels of growth have weak immune responses because of lower source of protein to produce antibodies (Pinard-van der Laan et al. 1998). Hyperpigmentation of the visceral peritoneum (HVP) was found to be influenced by genetic factors, with a heritability score of 0.33. HVP had positive genetic correlations with growth and carcass traits, such as leg muscle weight ($r_g = 0.34$), but had negative genetic correlations with immune traits, such as the antibody response to Newcastle disease virus ($r_g = -0.42$) (Luo et al. 2013). No significant genetic correlations were found between production, immune and disease traits in indigenous chickens, implying that selection for altered antibody response and/or disease resistance will not affect production (Psifidi et al. 2016).

Disease susceptibility or resistance has a genetic basis and can be inherited to the next generation. The availability of genome sequence and SNP opens the way to identify genes related to the immune system and health. Chicken genome has its own special characteristics that are different from those of mammals. Chicken genome is one-third of mammals’ in size because of reduced repetitive elements (Burt 2005). Genes responsible for resistance to diseases can be identified by linkage mapping analysis. Quantitative trait loci (QTLs) are the parts of DNA chain that are closely linked to the genes that control the traits of interest (Burt & Hocking, 2002). SNP map is an important tool to fine-map QTLs. Genetic diversity in chickens is more than that in humans with a high rate of SNP, about 5 SNPs per 1000 base pairs between and within lines (Van Hemert 2007). The analysis of SNP sequence data indicated that the variation between different avian breeds and those breeds with red jungle fowl as their nearest ancestor is similar. Around 70% of the SNPs were common to all breeds; therefore, it is concluded that most of the mutations originated before the domestication of chickens (Wong et al. 2004).

QTL analysis has been performed in chickens for many diseases such as Marek’s disease, salmonellosis, Newcastle disease, infection by E. coli and coccidiosis (Yonash et al. 2001; McElroy et al. 2005; Tilquin et al. 2005). QTL mapping for disease resistance is laborious, time-consuming and expensive because it needs extensive breeding to establish inbred lines with different traits. The resolution of QTLs found is limited and there are still many genes in these regions. In addition, different QTLs may be found for the same traits when different populations with different genetic variations are used (Lamont et al. 2008). Many of the QTLs, which are genome-wide significant or suggestive in the analyses of large intercross populations, are true effects that can be replicated and fine-mapped using repeated intercrossing of $F_2$ animals and successive generations (Besnier et al. 2011).

In the past decades, many studies have addressed the ability of candidate genes to improve resistance to SE in chickens. The results of association studies have indicated that SNPs at transforming growth factor β2 (TGFB2), TGFB3, toll-like receptor 4 (TLR4), inducible nitric oxide synthase (iNOS), natural resistance-associated protein1 (NRAMP1) and TNF-related apoptosis inducing ligand (TRAIL) genes were associated with SE burden in the caecum, spleen and liver of indigenous and commercial chickens (Beaumont et al. 2003; Tohidi et al. 2012, 2013a). Toll-like receptor 15 (TLR15) mRNA has been detected in chicken spleen, bursa, leukocyte, testis, ovary, sperm and bone marrow. This gene has not been identified in other vertebrates and, therefore, it is considered a chicken-specific gene (Nerren et al. 2009; Michailidis et al. 2011). Three SNPs were found in a single exon of chTLR15 and one of them results in an amino acid substitution. One SNP was associated with Salmonella natural infection status. The ‘T’ allele in C726T might be linked to resistance of Salmonella infection. The mRNA expression of TLR15 in heterophils of chickens infected with SE was lower than that of the control group at day 3 pi. However, TLR15 was up-regulated in the spleen of chickens infected by SE at day 3 pi (Hu et al. 2015). These genes are potential candidates for use in selection programmes for increasing genetic resistance against SE in chickens.

5. Gene expression and disease susceptibility

Pathogens can influence the expression of genes in the body of hosts. There are many strategies applied by bacteria to regulate the transcription of genes. Bacteria can modulate the signalling pathway of the host immune system to survive in the host cells (Hossain et al. 2006). Salmonella typhimurium can inhibit the expression of iNOS in host cells (Eriksson et al. 2000). The majority of genes affected by pathogens belong to the immune system. Infected cells on detection of pathogens make signals to trigger the immune system to react. The level of gene expression of the immune system is different between susceptible and resistant chickens. These differences are described in several studies. For example, resistant chickens to Marek’s disease had different gene expression in their lymphocytes compared to susceptible chickens (Liu et al. 2001). Table 1 indicates the gene expression status during treatments applied in previous studies. Expression of interleukin 2 (IL2), IL6, IL8 and Interferon γ (IFNγ) in the small intestine of chickens from a resistant line was higher than in those from a susceptible line (Rebel et al. 2005). The level of IL6, IL8 and IL18 mRNA in heterophils increased after resistant chickens were exposed to Salmonella, compared to susceptible chickens. Inversely, the level of TGFB4, an anti-inflammatory cytokine, decreased in heterophils derived from resistant chickens. Lower expression of IFNγ was observed in susceptible chickens to Salmonella than in resistant ones (Ferro et al. 2004; Swaggerty et al. 2004). Differences in gene expression depend not only on the existence of pathogens, but also on the status of the animal (i.e. age). Young chickens from a susceptible line had high levels of defensin gene mRNA under control conditions (Sadeyen et al. 2004). Salmonella enteritidis caused overregulation of iNOS, CXCL1, IL8, CCL3 and CCL4 in the oviduct of 25-week-old broiler hens (Li et al. 2009).

5.1. Gene expression response to Salmonella in intestine

Gastrointestinal tract is the first site that is infected by Salmonella. The number of different Salmonella serovars increased within 4 h to 4 days pi in caecal mucosa, epithelium and lamina propria of newly hatched chicks. At early stages of Salmonella infection, the innate immunity system including macrophages, granulocytes and immature dendritic cells is initiated. If Salmonella are still capable of surviving in macrophages, T cells
are promoted to move to the avian gut mucosa (Berndt et al. 2007; Van Hemert 2007). Early expression of cytokines, chemokines and apoptotic molecules in the chicken intestine has been reported in previous studies (Cheeseman et al. 2007; Van Hemert 2007; Tohidi et al. 2013b). The amount of CD4+ T-cells did not increase 1, 5 and 7 days pi with SE. However, the number of CD8+ T-cells increased 5 and 7 days pi. Only macrophages indicated a higher activity in the infected group on day 1 pi (Van Hemert 2007). CD4+ T-cells showed a plateau between 2 and 9 days pi. However, CD8+ increased during this time (Berndt et al. 2007). In newly orally infected hatched chicks with Salmonella, neither CD4+ nor γδ T-lymphocytes were found to be elevated in the jejunum. Inversely, CD8+ increased in the jejunum in response to Salmonella (Schokker et al. 2010). Infiltration of CD4+ T-cells in the ileum elevated on day 15 pi with S. typhimurium (Withanage et al. 2005). It can be concluded that CD4+ T-cells do not have a key role in eliminating Salmonella from the intestine before day 14 pi. However, it has been shown that CD4+ T-cells increased on day 7 pi in the thymus of one-day-old chickens inoculated with SE phage type 4 (Asheg et al. 2003). There was no significant increase in the number of CD4+ and CD8+ T cells from day 7 to 27 pi in the bursa of Fabricius. Furthermore, the actual number of CD4+ and CD8+ increased in peripheral blood on day 21 pi (Asheg et al. 2003). The maturation of T-lymphocytes is a reason for the late activation of these cells.

All of the immunological functions depend on gene transcripts. In recent years, many studies have been focused on the genetic responses to Salmonella infection. In the early gene expression studies, reverse transcriptase PCR and later real-time PCR were utilized to assay gene transcripts. However, too many genes have been analysed using

| Gene | Function | Organ | Treatment | Days post-infection | Fold change | Ref. |
|------|----------|-------|-----------|---------------------|-------------|------|
| K60  | Chemokine| Jejuna | S. typhimurium | 2 | >100 | Withanage et al. (2004) |
| Interleukin 8 | Chemokine | Jejuna | S. typhimurium | 2 | >10 | Withanage et al. (2004) |
| MIP-1 β | Chemokine | Ilea | S. typhimurium | 2 | >70 | Withanage et al. (2004) |
| Interleukin 8 | Chemokine | Liver | S. typhimurium | 2 | >1000 | Withanage et al. (2004) |
| Interferon γ | Cytokine | Liver | S. typhimurium | 7 | 50 | Withanage et al. (2005) |
| TGF-β4 | Cytokine | Spleen | S. typhimurium | 7 | >20 | Withanage et al. (2005) |
| Interleukin 6 | Cytokine | Caecum | S. typhimurium | 7 | 30 | Withanage et al. (2005) |
| Ras homolog gene family, member T1 | Response to Salmonella | Jejuna | S. enteritidis | 1 | 15 | Van Hemert (2007) |
| Dickkopf homolog 3 | Response to Salmonella | Jejuna | S. enteritidis | 1 | 14.2 | Van Hemert (2007) |
| Gag protein | Response to Salmonella | Spleen | S. enteritidis | 8 | 56.82 | Zhou and Lamont (2007) |
| ENV polyprotein | Response to Salmonella | Spleen | S. enteritidis | 8 | 39.9 | Zhou and Lamont (2007) |
| Natural resistance-associated macrophage protein1 | Divalent cation transporter | Caecum | S. enteritidis | 2 | 30 | Tohidi et al. (2013b) |
| Toll-like receptor 4 | Regulation of defence | Caecum | S. enteritidis | 2 | >80 | Tohidi et al. (2013b) |
| Matrix metallopeptidase7 | Degradation of extracellular matrix proteins | Caecum | S. enteritidis | 4 | 1430.8 | Rychlik et al. (2014) |
| ES1 protein homolog | Regulation of defence | Caecum | S. enteritidis | 4 | 28.3 | Volf et al. (2016) |
| Inducible NO synthase | NO radical production | Caecum | S. enteritidis | 4 | 37.09 | Rychlik et al. (2014) |
| Extracellular fatty acid binding protein | Fatty acid and bacterial siderophore binding | Caecum | S. enteritidis | 4 | 151.6 | Rychlik et al. (2014) |
| MRP-126, S100A9, calprotectin, calgranulin | Calcium and zinc binding | Caecum | S. enteritidis | 4 | 42.57 | Rychlik et al. (2014) |
| Serpin peptidase inhibitor | Protection of tissue against own proteases | Caecum | S. enteritidis | 4 | 30.95 | Rychlik et al. (2014) |
| Trappin 6-like | Protection of tissue against own proteases | Caecum | S. enteritidis | 4 | 36.46 | Rychlik et al. (2014) |
| Immune responsive gene1 | Itaconic acid and reactive oxygen species production | Caecum | S. enteritidis | 4 | 83.17 | Rychlik et al. (2014) |
| Serum amyloid A | Acute phase protein, LPS binding | Caecum | S. enteritidis | 4 | 84.63 | Rychlik et al. (2014) |
| Complement 3 | Complement | Caecum | S. enteritidis | 4 | 10.78 | Rychlik et al. (2014) |
| Avidin | Biotin binding, tissue reparation | Caecum | S. enteritidis | 4 | 15.15 | Rychlik et al. (2014) |
| Interleukin 1β | Cytokine | Caecum | S. enteritidis | 4 | 28.09 | Rychlik et al. (2014) |
| Lysozyme G-like2 | Antimicrobial peptide | Caecum | S. enteritidis | 4 | 37.21 | Rychlik et al. (2014) |
| Prostaglandin D2 synthase | Prostaglandin D2 synthesis | Caecum | S. enteritidis | 4 | 10.42 | Rychlik et al. (2014) |
| Glutamine γ-glutamyltransferase 4 | Protein crosslinking | Caecum | S. enteritidis | 4 | 24.63 | Rychlik et al. (2014) |
| Interleukin 22 | Cytokine | Caecum | S. enteritidis | 4 | 63.18 | Rychlik et al. (2014) |
| Interferon γ | Cytokine | Caecum | S. enteritidis | 4 | 32.08 | Rychlik et al. (2014) |
| NK-lysin | Lysis of own aberrant cells | Caecum | S. enteritidis | 4 | 16.73 | Rychlik et al. (2014) |
| Alcohol dehydrogenase 1B | Response to Salmonella | Caecum | S. enteritidis | 4 | −13.2 | Rychlik et al. (2014) |
high-throughput techniques. Some cytokines, chemokines and iNOS up-regulated 1 day pi with SE and *S. typhimurium* in the caecum of newly hatched chicks. Interferon γ, IL8, IL12, IL18, IL7Ra, MIP-1β, iNOS and LITAF were up-regulated in the caecum of the infected group rather than in the control chicken 1 day pi and commonly reached a peak 2 days pi. However, IL2 transcripts increased 4 days pi. Inversely, Fas and Bcl-x were down-regulated 4 days after infection (Berndt et al. 2007). Increased IL12 and IL18 that are produced by macrophages 2 days pi with *S. typhimurium* promote NK-cells to produce IFNy, a cytokine which strongly increases the influx of T-cells to lamina propria (Mastroeni et al. 1999; Cheeseman et al. 2007; Withanage et al. 2005). The up-regulation of IL8, MIP-1β, LITAF and iNOS within 12 h pi with *S. typhimurium* confirmed the role of proinflammatory cytokines and macrophages in eliminating *Salmonella* at the early stages of infection (Withanage et al. 2004; Cheeseman et al. 2007; Rychlík et al. 2014).

Data of microarray analysis revealed that 309 and 352 genes were significantly transcribed 12 and 24 h pi with SE in chicken caeca (Higgins et al. 2011). One of the most significant advantages of the high-throughput analysis is the creation of gene networks that show the global gene expression and relationship between the genes. Microarray analysis of mRNA transcripts isolated from 6-day-old chicken heterophils that were challenged with SE revealed that 115 and 48 genes up-regulated in the caeca (Higgins et al. 2011). One of the most significant advantages of the high-throughput techniques is the ability to identify complex cellular regulation. Luan et al. (2012) divided the 588 genes screened by the Agilent microarray into different categories according to their functions; for example, 24 genes associated with immune system process, 201 with metabolic process and 300 with cellular process. The genes that were identified to be up-regulated in response to the *Salmonella* infection in the intestine were involved in different stages of the immune responses in both chicken groups, such as TLR5, THBS1, KIT and FGF10. Toll-like receptor 5 (TLR5) is a signal transducer of several bacterial species and activates the innate immune system (Luan et al. 2012).

*Salmonella* is recognized by toll-like receptors in the caecum at the early stages of oral infection. This recognition induces chemokines and cytokines, and is followed by the expression of many functional genes, some of which are related to immune functions such as IL5, IFNγ and iNOS, and some are related to nutritional functions. Matrix metalloproteinase7 (MMP7) is expressed over 4000-fold during the first 10 days pi of newly hatched chicks. MMPs are a large family of zinc-endopeptidases which play important roles in multiple physiological and pathological processes (Fanjul-Fernández et al. 2010). Inducible immune responsive gene 1 (IRG1), serum amyloid A (SAA), extracellular fatty acid binding protein (ExFABP), serine protease inhibitor (SERPINB10), trappin 6-like (TRAP6), calprotectin (MRP126), mitochondrial ES1 protein homolog (ES1), interferon-induced protein with tetratricopeptide repeats 5 (IFIT5), aovidin (AVD) and transglutaminase 4 (TGM4) are the other functional genes that are highly induced after SE infection in the caecum (Rychlík et al. 2014). Using a combination of 454 pyrosequencing, protein mass spectrometry and quantitative real-time PCR, Matulova et al. (2013) identified 48 down- and 56 up-regulated chicken genes after SE infection. The most inducible gene was that coding for MMP7, exhibiting a 5952-fold induction 9 days pi. Since prostaglandin D2 synthase was up-regulated and degrading hydroxy prostaglandin dehydrogenase was down-regulated after the infection, prostaglandin must accumulate in the caecum of chickens infected with SE. Furthermore, signaling was dependent on the presence of a SPI1-encoded type III secretion system in SE. The inflammation lasted for 2 weeks, after which time the expression of the ‘inflammatory’ genes returned back to basal levels and, instead, the expression of IgA and IgG increased. This points to an important role for immunoglobulins in the restoration of homeostasis in the caecum after infection (Matulova et al. 2013).

Duration of exposure to infection may affect the regulation of genes. The transcript level of Sin3A-associated protein (SAP30) was different in the caecum of one-day-old chickens that were treated with SE 12 and 24 h post-challenge. Pathway analysis indicated that genes associated with the nuclear factor kappa B complex as well as apoptosis were regulated during infection with *Salmonella* in the chicken caecum (Higgins et al. 2011).

### 5.2. Gene expression in systemic organs

Three separate phases for avian systemic salmonellosis are generally assumed. The first phase is the invasion of *Salmonella* into the GIT. The second phase is the establishment of infection in the macrophages. In the third phase, the immune system may overcome the infection and clear it completely; otherwise, the bird may die or the subclinical phase develops (Chappell et al. 2009). *Salmonella* are picked up by macrophages and dendritic cells following invasion into the GIT and then the spleen and liver through the lymphatic system (Mastroeni & Menager 2003). SE colonizes in the chicken caecum 6–12 h after inoculation, whereas bacteria proliferate in the spleen and liver 1–2 days pi (Van Immerseel et al. 2002; Withanage et al. 2004).

Previous studies indicated that the kind and level of gene expression in the spleen and liver may be different from those expressed in the caecum. Interferon γ transcripts were higher in the spleen than in the caecum of chickens inoculated with SE 18 h pi (Cheeseman et al. 2007). However, Nramp1 was commonly increased in the caecum and spleen of SE-challenged chickens 48 h pi, whereas TLR4, IL8 and IFNy were only up-regulated in the caecum at the same time (Tohidi et al. 2013b). Microarray analysis revealed 272 genes that were significantly up-regulated on day 7 pi with SE and 490 genes on day 8 in high splenic SE burden compared to unchallenged chickens. Among the genes, chemokine ah294, Quiescence-specific protein precursor, Serum amyloid type A, Gag protein, ENV polyprotein and Avidin precursor were expressed more than four-fold (Zhou & Lamont 2007). The genes involved in proinflammatory response as well as apoptosis have an important role in initiating immune functions against *Salmonella*. Interleukin 8, MIP-1β, CXCR4, TNF receptor,
Caspase1, 2 and 8, Interferon regulatory factor 1 (IRF1), IRF4, IL-1 receptor-associated kinase, TGFβ3 and IL-1β have been shown to increase in the spleen of SE-infected chickens (Withanage et al. 2004; Zhou & Lamont 2007). The expression of CXC chemokines K60, IL-8 and MIP-1β was greater in the liver of SE-infected chickens 12 h pi, whereas IL-1β increased 24 h pi (Withanage et al. 2004).

High-throughput technology revealed several genes highly expressed in infected birds. However, the exact role of many genes is not known yet. The complicated interaction between the immune system and metabolism contributes to the immune responses to SE inoculation of egg-type chickens at 14 dpi at the onset of lay. Functional annotation revealed that several GO terms related to immunity were significantly enriched between the inoculated and non-inoculated groups at 14 dpi but not at 7 dpi. Glucocorticoid (GC), TNFSF8, CD86, CD274, BLB1 and BLB2 play important roles in response to SE inoculation. TNFSF8, also known as CD30, is a membrane-associated glycoprotein belonging to the TNF superfamily (TNFSF). The TNFSF8 gene plays an important role in the induction of apoptosis (Wu et al. 2015). GC hormones play a critical role in immunomodulatory processes as well as in T and B lymphocyte development and selection (Lechner et al. 2001).

6. Conclusion

Resistance to disease is a quantitative trait that is controlled by several genes and environmental conditions. By now, a huge number of genes involved in immune function have been identified. Application of new technologies such as next-generation sequencing, RNA-seq, microarray expression analysis and high-density SNP genotyping aid us to map a huge quantity of genes and to identify SNPs influencing resistance to Salmonella. The important matter is how these genes should be used in marker-assisted selection. Some genes are more active in the caecum and some in the spleen as well as in the liver. This activity also depends on the age of chicken, duration of infection, correlation between genes and environment. It is clear that some genes such as ILS, IFNγ, TLRs, iNOS and genes involved in apoptosis directly influence resistance to Salmonella infection. But still, it is not easy to make a decision about what kind of genes should be chosen in a special selection programme.

As the intestine is the first place for colonization of Salmonella, the genes that are more active in the caecum are more preferable in a selection programme. A network analysis system including both genomic information and phenotypic records would be more effective in finding causal immune-related genes. However, environmental conditions should be considered in the analysis. Therefore, a global pattern accompanied by a local analysis could be an appropriate solution to establish an effective selection programme. Having a unique strategy regarding the age of animals at the beginning of the study and duration of a treatment applied is necessary to make a general protocol for a selection programme.

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No potential conflict of interest was reported by the authors.

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