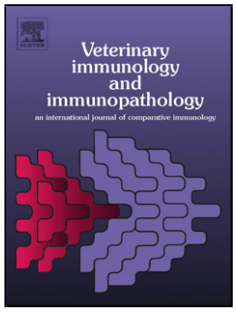

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Global gene expression analysis of chicken caecal response to *Campylobacter jejuni*

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Abstract

Campylobacter jejuni colonises the caecum of more than 90% of commercial chickens. Even though colonisation is asymptomatic, we hypothesised that it is mediated by activation of several biological pathways. We therefore used chicken-specific 20K oligonucleotide microarrays to examine global gene expression in *C. jejuni*-challenged birds. Microarray results demonstrate small but significant fold-changes in expression of 270 genes 20 hours post-challenge, corresponding to a wide range of biological processes including cell growth, nutrient metabolism and

immunological activity. Expression of *NOX1* (2.3-fold) and *VCAM1* (1.5-fold) were significantly increased in colonised birds ($P<0.05$), indicating oxidative burst and endothelial cell activation, respectively. Microarray results, supplemented by qRT-PCR analyses demonstrated increased *TOPK* (1.9-fold), *IL17* (3.6-fold), *IL21* (2.1-fold), *IL7R* (4-fold) and *CTLA4* (2.5-fold) gene expression ($P<0.05$), which was suggestive of T cell mediated activity. Combined these results suggest that *C. jejuni* has nominal effects on global caecal gene expression in the chicken but significant changes detected are suggestive of a protective intestinal T cell response.

Keywords: Chicken; Commensal; *Campylobacter*; Global gene expression; Intestinal immune response.

1. Introduction

Campylobacter jejuni is responsible for the majority of gastroenteritis cases in humans (Poly and Guerry, 2008; Young et al., 2007). More than 90% of commercial chickens are colonised by the microbe (Newell and Fearnley, 2003), particularly within the lower gastrointestinal tract (Beery *et al.*, 1988). Contamination of poultry meat occurs easily during slaughter and this together with the popularity of poultry products, has led to chickens becoming the principal vector for *C. jejuni* infection in humans (Humphrey *et al.*, 2007).

Mammalian studies have shown that local immune responses are important regulators of bacterial colonisation in the intestine and involve strategies to eliminate microbial colonisers while preventing excessive inflammation. Engagement of microorganisms with pattern recognition receptors (PRRs) of epithelial cells results in the production of peptides with broad-spectrum antimicrobial activity (Petnicki-Ocwieja *et al.*, 2009; Salzman *et al.*, 2010). Proinflammatory cytokine and chemokine production is often stimulated, which serve to destroy and contain microbial species but may also contribute to intestinal inflammation (Magalhaes *et al.*, 2007). Anti-inflammatory responses are also characteristic, and are key to preventing excessive intestinal pathology (Tsuji and Kosaka, 2008). Avian proinflammatory and anti-inflammatory intestinal responses appear to be similar to their mammalian counterparts (Brisbin *et al.*, 2008; Chappell *et al.*, 2009) although important differences in immune gene repertoires are evident (Cormican *et al.*, 2009; Higgs *et al.*, 2006; Kaiser, 2007). Some of these differences may explain why *C. jejuni* is a pathogen in humans but commonly regarded as a commensal in birds.

To date, the avian innate immune response to *C. jejuni* in the caecum has not been extensively studied, with two studies showing significant increases in chemokine but not cytokine expression. In newly-hatched and 2-week-old chickens, *K60* (*CXCLi1*) and *IL8* (*CXCLi2*) gene expression is increased but *IL1β* and *IL6* gene

expression increases were minor (Smith *et al.*, 2008). Similarly, in 4-week-old birds, high *IL8* but low *K60*, *IL1β and IL6* gene expression increases were demonstrated while *IFNG* was not differentially expressed (Shaughnessy *et al.*, 2009). It is still unclear whether the lack of cytokine gene expression increases is a reflection of *C. jejuni*-mediated subversion of the innate immune response, novel host-immunoregulatory processes or failure of avian PRRs to recognise the microbe.

Microarrays have previously been used to investigate the avian response to various microbial species. Interestingly, the immune response seems to figure extensively in most of these studies and the microarray approach has led to some success in characterising local immune responses. In chicken heterophils, several TLR signalling components and proinflammatory effector molecules were differentially expressed in response to *Salmonella enterica* serovar Enteritidis (Chiang *et al.*, 2008) while multiple innate and adaptive immune components were increased in response to *Clostridium perfringens* in the spleen (Sarson *et al.*, 2009). Furthermore, microarrays proved useful for differentiating between primary and secondary intestinal immune responses to *Eimeria* species (Min *et al.*, 2003). Recently, global gene expression analyses on the caeca of two different chicken lines revealed decreased *IL1β*, *IL8*, *K60* and *SOCS3* gene expression in response to *C. jejuni* at seven days post-challenge (Li et al., 2010).

The aim of this study was to use whole-genome microarray to examine the early global gene expression profile of *C. jejuni* colonised caeca of 4-week-old commercial broiler chickens at 20 hours post-challenge.

2. Materials and Methods

2.1 Bacterial strain preparation and experimental challenge

Campylobacter jejuni strain NCTC11168 v1 was grown on Meuller Hinton (MH) agar plates. The cells were resuspended in saline and viable bacterial numbers were determined by plating serial dilutions on MH agar plates (2.5×10^8/ml). The *C. jejuni* challenge model was performed as previously described (Meade *et al.*, 2008). For the analysis of the caecal transcriptional response to *C. jejuni* challenge, 4-week-old birds were used from this study representing seven mock-challenged (20 hours post-challenge), seven *C. jejuni*-challenged (20 hours post-challenge) and eight unchallenged birds.

2.2 RNA extraction, cDNA synthesis and labelling, and microarray experimental design

Total RNA was extracted from caecum samples using an RNeasy® Mini Kit (Qiagen) according to manufacturer's instructions. Additionally, all samples were DNase treated with the RNase-Free DNase Set (Qiagen). RNA yield and quality was then assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies). All RNA had RNA integrity numbers (RIN) greater than 9.

Gene expression between *C. jejuni*-challenged and mock-challenged birds was compared using a common-reference design (CRD), which involves hybridising each different biological sample to a common sample (Churchill, 2002). The common reference sample consisted of RNA pooled from the caeca of unchallenged birds. This design involves hybridizing the common reference sample with both the *C. jejuni*-challenged and mock-challenged samples enabling flexible data comparisons, and avoiding artificial direct pairings between animals from each treatment group. To achieve this, 8 µg of common reference RNA and 10 µg sample RNA were reverse

transcribed into cDNA using the Superscript ™ Plus Direct cDNA Labelling System (Invitrogen) according to manufacturers instructions. The common reference was labelled with Alexa Fluor® 647 while the challenge/mock-challenged samples were labelled with Alexa Fluor® 555. cDNA concentrations and dye incorporation were determined using a Nanodrop™ 1000 Spectrophotometer (Thermo Scientific).

Chicken specific oligonucleotide microarrays containing 24,182 (20K) genes/ transcripts, supplied by Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, were used in this study. The gene/transcript library was similar to the ARK-Genomics *G. gallus* 20K v1.0 library (www.ark-genomics.org/microarrays/bySpecies/chicken) except the arrays were spotted by a gene array facility located in the Prostate Centre at Vancouver General Hospital (www.microarray.prostatecentre.com).

2.3 Microarray hybridizations and array scanning

Each labelled reference sample was combined with a labelled sample in addition to 90 µl SlideHyb Glass Hybridization Buffer #3 (Ambion Inc.) to form the probe. 100µl of probe was hybridised to 20K oligonucleotide chicken microarrays using the following program on an automated HS 400™ Pro Hybridization Station (Tecan UK Ltd.) – WASH (4X SCC/ 0.2% SDS): 75°C, Runs 1, Wash 10 s, Soak 20 s; PROBE INJECTION: 85°C, Denaturation: 95°C, 2 min; HYBRIDISATION: 42°C, Agitation Frequency Medium 4 hours; HYBRIDISATION: 35°C, Agitation Frequency Medium 4 hours; HYBRIDISATION: 30°C, Agitation Frequency Medium 4 hours; WASH (2X SCC/ 0.1% SDS): 37°C, Runs 2, Wash 10 s, Soak 20 s; WASH (0.2X SCC/ 0.1% SDS): 25°C, Runs 2, Wash 15 s, Soak 30 s; WASH (0.2X SCC): 25°C, Runs 2, Wash 20 s, Soak 40 s and SLIDE DRYING: 25°C, 2 min N_2 at 2.7 bar.

Each array was subsequently scanned using a Genepix 4000B scanner (Molecular Devices Ltd.).

2.4 Data pre-processing, normalization and analysis

The median background intensities were subtracted from the median foreground intensities to establish the working signal intensities for subsequent analysis using BRB-ArrayTools version 3.8.0 (Simon *et al.*, 2007). Normalizations and data analyses were also carried out using BRB-ArrayTools. The raw data was adjusted using Median (Zien *et al.*, 2001), locally weighted scatterplot smoothing (Lowess) and Print-Tip Lowess normalizations (Yang *et al.*, 2002). Prior to and following normalizations the data was visualised using MA plots, which plot the difference in average log intensities against the average of log intensities ($M = \log_2 R - \log_2 G$; $A = ½(\log_2 R + \log_2 G)$)(Yang *et al.*, 2002). Class comparisons (univariate parametric and non-parametric tests) between the *C. jejuni*-challenged and mock-challenged data sets were subsequently carried out to compare the three normalization strategies. To calculate the false discovery rate (FDR), class comparisons were repeated with randomly classified array data sets. On the basis of MA-plot symmetry and the FDR, Lowess was deemed the most appropriate normalization strategy.

2.5 Validation of differentially expressed genes

Following class comparisons, genes with alpha levels of $P<0.01$ were deemed significantly differentially expressed. The 70 nucleotide probe of each differentially expressed gene was compared against the chicken genome using BLAST (basic local alignment search tool), to initially annotate the genes. GO Slim using the Protein Information Resource (PIR) subset of GO terms was used to assign known biological functions to each significantly differentially expressed gene. Eight significant genes

associated with the immune response were subjected to validation via qRT-PCR. Immune gene validation was extended to two significant genes identified through Median and Print-Tip Lowess normalizations. Validation revealed evidence of T cell activity and as a result, further genes indicative of such activity were also analysed. Primer design and cDNA synthesis were carried out as previously described (Shaughnessy *et al.*, 2009). A total of 40 ng cDNA, quantified using a NanoDrop® ND-1000 spectrophotometer, was subsequently used for each real time qRT-PCR reaction. Each reaction was carried out in duplicate, in a total volume of 25 µl with 2 µl of cDNA (20 ng/µl), 12.5 µl PCR master mix (Stratagene Corp, La Jolla, CA) and 10.5 µl primer/H_2O. Real time qRT-PCR was performed using a MX3000P® quantitative PCR system (Stratagene Corp, La Jolla, CA) using the following cycling parameters: 95°C for 30 s. 60°C for 1 min and 72°C for 30 s followed by amplicon dissociation. All gene amplifications were normalised to Ribosomal protein L7 (*RPL7*) gene expression. Primer sets for each gene analysed are described in supplementary table 3.

3. Results

3.1 Comparison of normalization strategies

Different normalization strategies account for different aspects of technical variation that occur during microarray data generation. Choosing the most appropriate normalization approach was necessary for gaining an accurate representation of the gene expression datasets and ratios between *C. jejuni*-challenged and mock-challenged birds. Initially, the data from each of the hybridizations was visualised using MA-plots. With the raw data, asymmetrical plots were evident, which suggested intensity based dye biases (Supplementary fig. 1; panel 1). Median, Lowess and Print-Tip Lowess normalizations (Smyth and Speed, 2003) were applied to the data to determine their effects on the raw data. Asymmetrical MA-plots were retained following Median normalization indicating inappropriate normalization (Supplementary fig. 1; panel 2). In contrast, the application of Lowess and Print-Tip Lowess normalizations resulted in symmetrical MA-plots (Supplementary fig.1; panel 3 and 4 respectively), which indicated successful removal of intensity related fluorescent dye bias.

Different normalization methods can result in varying numbers of significantly differentially expressed genes, and can influence the numbers of false-positive and false-negative genes discovered. Significantly differentially expressed gene lists resulting from each of the three normalization strategies used were compared for overlapping genes. At the $P<0.01$ level, 250, 270 and 188 genes were significantly differentially expressed using Median, Lowess and Print-Tip Lowess normalizations respectively (Supplementary fig. 2A and 2B). Only 64 genes were common to all three methods, although there was strong overlap in resulting genesets (193 genes) between Median and Lowess normalizations (Supplementary fig. 2A).

To gain an insight into the false discovery rate (FDR) for each of the normalization strategies, *C. jejuni*-challenged and mock-challenged microarrays (n=14) were randomly classified so that classes consisted of both *C. jejuni*-challenged and mock-challenged arrays. Class comparison was subsequently performed and in this regard, significantly differentially expressed genes identified would be expected to represent false-positive genes. For Median, Lowess and Print-Tip Lowess normalizations, 88, 84 and 103 falsely discovered genes were evident respectively. When compared to the analyses based on true classifications, FDRs of 0.35, 0.31 and 0.54 respectively were apparent (Supplementary fig. 2B). Considering, the lower FDR and the appropriate MA-plot adjustments, Lowess was chosen as the most appropriate normalization strategy for subsequent reliable determination of true significant differential gene expression. Lowess normalization applies a local regression curve to the raw data that addresses intensity-dependent dye biases (Yang *et al.*, 2002).

3.2 Characterisation of global differential gene expression in response to C. jejuni

Global gene expression was compared between *C. jejuni*-challenged and mock-challenged birds at 20 hours post-inoculation. This timepoint previously revealed evidence of high *IL8* gene expression in response to *C. jejuni* (Shaughnessy *et al.*, 2009) and thus was chosen as an appropriate timepoint for in-depth microarray analysis. At the $P<0.01$ level, 270 genes were significantly differentially expressed between the two groups with only minor fold changes evident. Initial characterisation involved 70 nucleotide probe sequences corresponding to differentially expressed genes being aligned against the chicken genome using BLAST. Fifty-three hits corresponded to hypothetical proteins, 115 hits mapped to poorly annotated regions of the genome while 102 hits corresponded to known or predicted genes (Supplementary table 1). Of the annotated genes, 17 were increased greater than 1.5-fold (Table 1) and 25 were decreased by more than 1.5-fold (Table 2).

GO Slim using the Protein Information Resource (PIR) subset of GO terms was used to provide gene ontology for the significantly differentially expressed genes. A wide range of biological processes were evident (supplementary table 2 and fig. 1). Metabolic activities were highly represented such as macromolecule metabolic processes (GO:0043170; 159 gene products) and nitrogen compound metabolic processes (GO:0006807; 102 gene products). Immune response terms were also identified, namely immune system processes (GO:0002376; 21 gene products) and cytokine production (GO:0001816; 6 gene products). In total, 104 biological processes correlated to the differentially expressed data (supplementary table 2).

3.3 Validation of significantly differentially expressed genes

Differentially expressed genes associated with immunological processes were chosen for validation by qRT-PCR. Of eight genes investigated, four were confirmed to be significantly differentially expressed, indicating a 50% correlation between microarray and qRT-PCR analyses (Fig. 2). Expression of genes encoding Vascular cell adhesion molecule 1 (1.5-fold; P=0.002) and NADPH oxidase 1 (2.3-fold; P=0.002) were increased while mitogen-activated protein kinase 9 (-1.3-fold; P=0.002) and C1q tumor necrosis factor related protein 7 (-3.19-fold; P=0.025) were decreased in *C. jejuni* challenged birds.

As normalization occasionally can remove truly differentially expressed genes (false negatives), immune genes identified through the other normalization approaches were also potentially significant. Specifically, there was evidence of T cell activity in the immune response to *C. jejuni* colonisation and such genes were also validated. Significantly increased *TOPK* (1.9-fold; P=0.025), *IL17* (3.6-fold; P=0.002) and *IL21* (2.1-fold; P=0.025) gene expression was evident in response to *C. jejuni* in the chicken caeca (Fig. 3). Additional genes associated with T cell activity that were not represented on the microarray platform were also investigated via qRT-

PCR. Consequently, significantly increased *KK34* (1.64-fold; *P*=0.004), *IL7R* (3.97-fold; *P*=0.003) and *CTLA4* (2.54-fold; *P*=0.003) gene expression was identified in *C. jejuni* challenged birds.

4. Discussion

C. jejuni is highly prevalent in chicken flocks, often resulting in infection rates as high as 90% (Evans and Sayers, 2000). While *C. jejuni* rarely causes pathology in chickens, it is the principal human bacterial enteropathogen, causing more cases of gastroenteritis incidences than *Salmonella* spp. and *Escherichia coli* combined (DuPont, 2007; Wysok and Uradzinski, 2009). In this regard, reducing *C. jejuni* carriage in chickens would indirectly reduce human infections. There is evidence that chicken host genetic factors influence *C. jejuni* levels, particularly at the primary site of colonisation, the caeca (Boyd *et al.*, 2005; Li *et al.*, 2008). However the precise factors regulating colonisation, and the immune response to *C. jejuni* has been poorly characterised in chickens, to date. In order to identify such processes, we used whole-genome microarrays to examine global gene expression in the caecum of *C. jejuni*-challenged and mock-challenged chickens at 20 hours post-inoculation.

In order to obtain an accurate representation of differential gene expression between the *C. jejuni*-challenged and mock-challenged cohorts, the data was normalized. For the identification of the most appropriate adjustment strategy, Median, Lowess and Print-tip Lowess normalizations were initially applied to the data. These normalization strategies address dye biases, intensity-dependent dye biases and spatial-dependent dye biases, respectively (Yang et al., 2002; Zien et al., 2001). Applying Median normalization to the data resulted in asymmetrical MA plots. In contrast, both Lowess and Print-tip Lowess normalizations induced symmetrical MA plots. MA plot symmetry is associated with appropriate data adjustment (Yang et al., 2002) and thus, it appeared that applying the Lowess normalization methods produced reliable datasets. Surprisingly however, there was a poor correlation between gene lists arising from Lowess and Print-tip Lowess normalizations. Furthermore, Lowess Print-tip normalization was found to have the highest FDR (0.54). There was, however, a strong correlation between the datasets generated by

Lowess and Median normalization (193 differentially expressed genes in common) and as a result, Lowess was chosen as the most appropriate normalization strategy.

Twenty hours post-challenge, 270 genes were differentially expressed in the caeca of *C. jejuni* colonised chickens. Functional annotation of the dataset using Gene Ontology (GO) revealed minimal activation of a wide variety of biological processes including cell growth, neurological response, nutrient metabolism and immune response (Fig. 1). Interestingly, Li *et al.*, 2010 also observed the latter two processes in the caeca of *C. jejuni*-challenged broiler chickens at 7-days post-challenge (Li et al., 2010). Thus, it would appear that such processes are activated immediately following challenge and also several days post-colonisation. While it is possible that the introduction of high *C. jejuni* levels have a profound impact on caecal metabolic events, the intestinal immune response processes identified are likely to be of greater importance, considering that intestinal defences are regarded as key regulators of bacterial colonisation (Duerkop *et al.*, 2009). We thus focused our subsequent analysis on differentially expressed genes associated with immunological processes.

Previous innate immune gene expression profiled using qRT-PCR revealed high *IL8* with minor *IL1β* and *K60* increases between *C. jejuni*-colonised and uncolonised birds at 20 hours post-challenge (Shaughnessy *et al.*, 2009). Surprisingly, microarray analysis of RNA from the same birds and timepoint, did not reveal differential expression of these genes. Several reasons may explain these false-negatives: cDNA corresponding to some genes may fail to hybridize to their respective probes (Lee *et al.*, 2000); microarrays have limited detection of lowly abundant mRNA (Venkatasubbarao, 2004), which generally include cytokines; and normalization sometimes removes truly differentially expressed genes (Eddy and Storey, 2008). To explore the latter possibility, datasets normalised using Median and Print-tip Lowess normalizations were also investigated for evidence of *IL8* expression. *IL1β, IL8* and *K60* were not differentially expressed in any dataset

resulting from the three normalisation methods. Furthermore, as microarray fold-changes were low albeit significant, such changes must be interpreted with caution and qRT-PCR confirmation and validation of results should be used. Interestingly, Li et al also reported somewhat high FDRs (ranging from 0.17 to 0.58) and low differential gene expression changes in response to *C. jejuni* in the caeca (Li et al., 2010). Genetic variability contributing to significant inter individual variation in host response may obscure changes in genes expressed at low levels, and is potentially a limitation to such global analysis approaches. On the other hand, minimal global gene expression changes may be somewhat expected, considering that *C. jejuni* and birds have co-evolved towards a commensal relationship. We observed minimal proinflammatory gene expression increases, despite our *C. jejuni* challenge dose being approximately 700 times greater than the dose used by Li *et al.*, 2010.

Nevertheless, through microarray analysis, several immune genes were shown to be differentially expressed and were selected for qRT-PCR validation. Of eight genes selected, only four were confirmed to be differentially expressed. These included NADPH oxidase 1 (*NOX1*), which is associated with the production of reactive oxygen species from phagocytic cells (Sumimoto, 2008); vascular cell adhesion molecule 1 (*VCAM1*), a marker for endothelial activation (Wittchen, 2009); inhibitor of kappa light polypeptide gene enhancer in B-cells (*IKBKB*), a component of NF-κB signalling (Rothwarf *et al.*, 1998); and complement-C1q tumor necrosis factor-related protein isoform 2 (*C1QTNF2*).

Collectively between the microarray data sets, there was evidence of T cell activity in response to *C. jejuni*. Validation of such genes revealed minor lymphokine-activated killer T cell-originated protein kinase (*TOPK*), *IL17* and *IL21* gene expression increases. *TOPK* is expressed by activated T lymphocytes (Abe *et al.*, 2000), while IL-17 and IL-21 are cytokines produced by such cells (Bhave and Carson, 2009; Miossec et al., 2009). To further investigate such responses, key

markers associated with proinflammatory and anti-inflammatory T cell activity were also profiled by comparing *C. jejuni*-challenged and mock-challenged birds by qRT-PCR. IL7Rα (CD127) and CTLA4, receptors expressed on the surface of various T cell types (Racape *et al.*, 2009; Rudd *et al.*, 2009), were significantly increased at the mRNA level. Interestingly, IL7Rα facilitates the proliferation of T lymphocytes (Racape *et al.*, 2009) and has been shown to be differentially expressed by activated avian $CD4^+$ and $CD8^+$ T cells (van Haarlem et al., 2009). On the other hand, CTLA4 regulates proinflammatory responses (Rudd *et al.*, 2009). In this regard, proinflammatory T cell responses would regulate *C. jejuni* colonisation while anti-inflammatory T cell responses might reduce immunopathology. Such responses are likely to remain evident throughout colonisation, considering that a recent global gene expression analysis study has revealed evidence of lymphocyte activation in *C. jejuni*-colonised caeca at 7-days post-inoculation (Li et al., 2010).

In the current study, we can hypothesise that our data points toward an innate T cell responses, which is more likely than an adaptive T cell responses, considering the timepoint is 20 hours post-challenge in birds which have not previously encountered *C. jejuni*. γδ T cells are abundant in the chicken caecum and mediate responses towards intestinal bacteria (Berndt *et al.*, 2007). Mammalian γδ T cells have been shown to produce IL-17 (O'Brien *et al.*, 2009; Sutton *et al.*, 2009) while other γδ T cell subsets have been shown to limit pathology in the gut (Ziegler, 2004). Hence, γδ T cell populations may have an important role in *C. jejuni* colonisation in the chicken caeca.

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Figure captions

Fig. 1. GO Slim using the Protein Information Resource (PIR) subset of GO terms reveals that global gene expression in response to *C. jejuni* in the caeca correlates to a wide range of biological processes. A selection of such processes is represented above (see supplementary table 2 for complete list). Gene product numbers are a representation of the number of differentially expressed genes within each biological process category.

Fig. 2. Validation of immune gene expression via qRT-PCR. The horizontal line within each box plot represents the median fold changes in caecal gene expression in *C. jejuni*-challenged chickens relative to mock-challenged controls (*P<0.05, **P<0.01).

Fig. 3. T cell related gene expression in response to *C. jejuni* in the chicken caeca at 20 h. The horizontal line within each box plot represents the median fold changes in caecal gene expression in *C. jejuni*-challenged chickens relative to mock-challenged controls(*P<0.05, **P<0.01).

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Table 1.

List of significantly increased (**>1.5-fold**) genes (*P*< 0.01) in the caeca between *C. jejuni*-challenged (n =7) and mock-challenged chickens at 20 hours (n =7). List was generated using Lowess normalization followed by class comparison (BRB Array tools). See **supplementary table 1** for significantly increased genes <1.5-fold.

P-value	***Fold-change***	**Gene description**
0.0022	1.5	LIM domain kinase 2
0.0027	2.2	Vitellogenin binding protein (VBP), beta/beta isoform
0.003	1.5	Cullin 2
0.0039	1.5	Similar to splicing factor Prp8
0.0042	1.5	Similar to MLTK-alpha
0.0043	2	Similar to potassium voltage-gated channel subfamily E member 1
0.0044	1.5	Similar to solute carrier family 39 (zinc transporter)
0.0046	1.5	Similar to histone stem-loop binding protein
0.0061	1.9	Similar to Transforming growth factor, beta receptor associated protein 1
0.0086	2.6	Similar to axonemal heavy chain dynein type 3
0.0087	2.3	Zinc finger protein 367
0.0088	2.7	Similar to G-protein coupled receptor (putative)
0.0089	2.3	Similar to progestin and adipoQ receptor family member III
0.0089	1.6	Similar to bactericidal/permeability-increasing protein-like 2
0.0091	1.5	Similar to ubiquitin specific peptidase 3
0.0091	1.5	Chimerin (chimaerin) 1
0.0092	1.8	Zinc finger protein 289, ID1 regulated

Table 2.

List of significantly decreased (**>1.5-fold**) genes ($P< 0.01$) in the caeca between *C. jejuni*-challenged (n =7) and mock-challenged chickens at 20 hours (n =7). List was generated using Lowess normalization followed by class comparison (BRB Array tools). See **supplementary table 1** for significantly decreased genes <1.5-fold.

P-value	***Fold-change***	**Gene description**
0.0014	-1.5	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
0.0016	-1.5	Resistance to inhibitors of cholinesterase 8 homolog A
0.0019	-1.9	H3 histone, family 3B
0.0021	-1.7	Similar to Nedd4 binding protein 2
0.0022	-1.6	Similar to Leucine-rich repeats and calponin homology (CH) domain containing
0.0027	-1.6	Similar to chromosome X open reading frame 20
0.0029	-1.5	Similar to ganglioside sialidase
0.003	-1.6	Similar to vasculin
0.0038	-1.9	Similar to REX1, RNA exonuclease 1 homolog (S. cerevisiae)
0.004	-1.6	Similar to low density lipoprotein receptor-related protein 6
0.0052	-1.5	TXK tyrosine kinase
0.0052	-1.7	Similar to group IIE secreted phospholipase A2
0.0055	-1.5	LOC396098
0.0056	-1.7	Zinc finger protein 593
0.0062	-1.7	Similar to Tubulin alpha-3/alpha-7 chain
0.0068	-1.5	Similar to nuclear receptor co-repressor 1
0.0068	-2.1	Similar to vacuolar protein sorting 13B
0.0069	-1.9	Similar to neural visinin-like Ca2+-binding protein type 2
0.007	-1.6	Similar to Na+-dependent purine-selective nucleoside transporter
0.0072	-1.8	Similar to chromosome 9 open reading frame 5
0.0081	-1.9	Similar to hyaluronic acid synthase 3
0.0084	-1.5	Similar to solute carrier family 30 (zinc transporter), member 8
0.0084	-1.5	Similar to patched-2
0.0086	-1.9	Similar to cytidine monophospho-N-acetylneuraminic acid synthetase
0.01	-1.6	Similar to polypeptide N-acetylgalactosaminyltransferase 17

Figure 1

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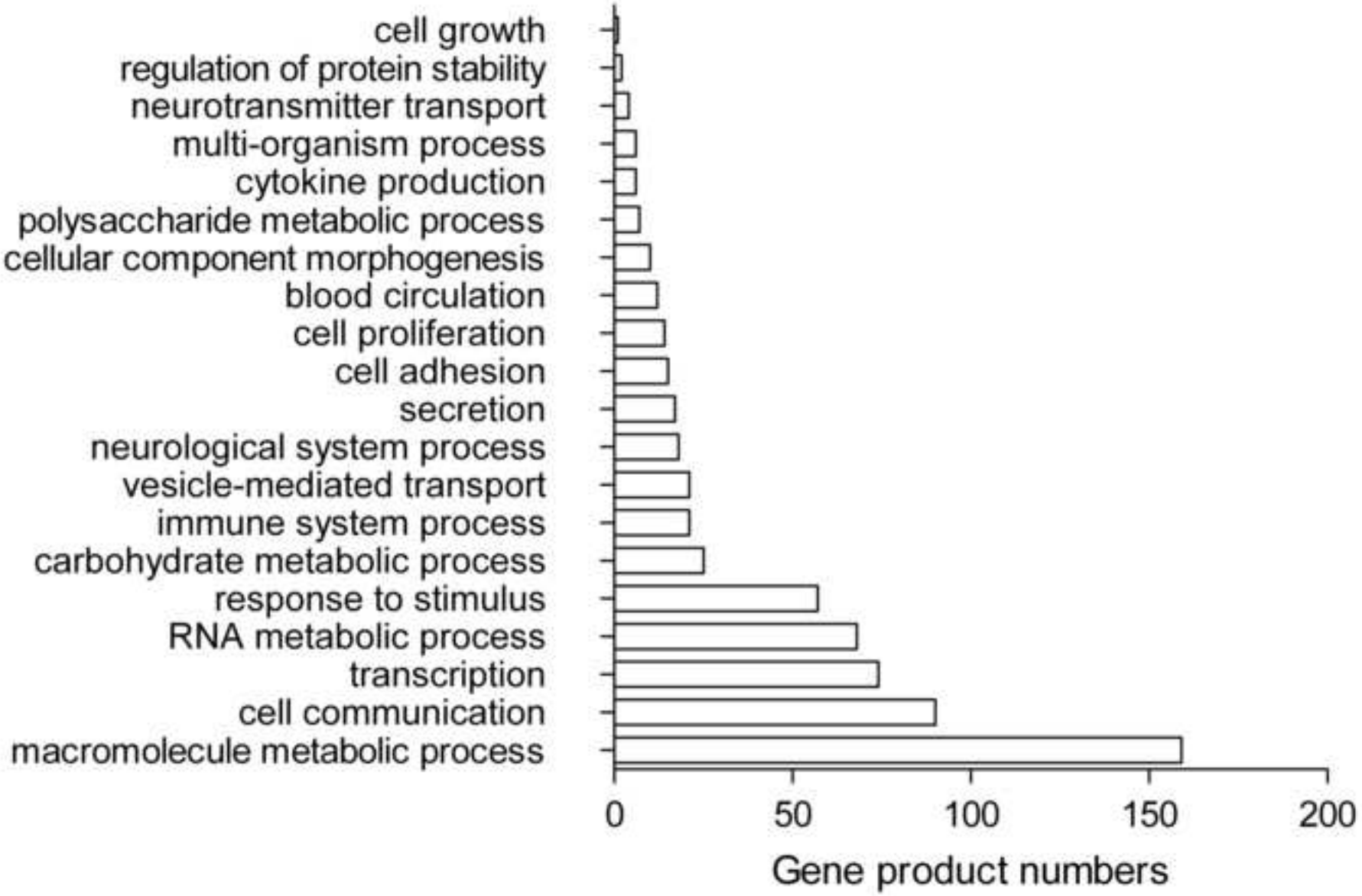

Figure 2

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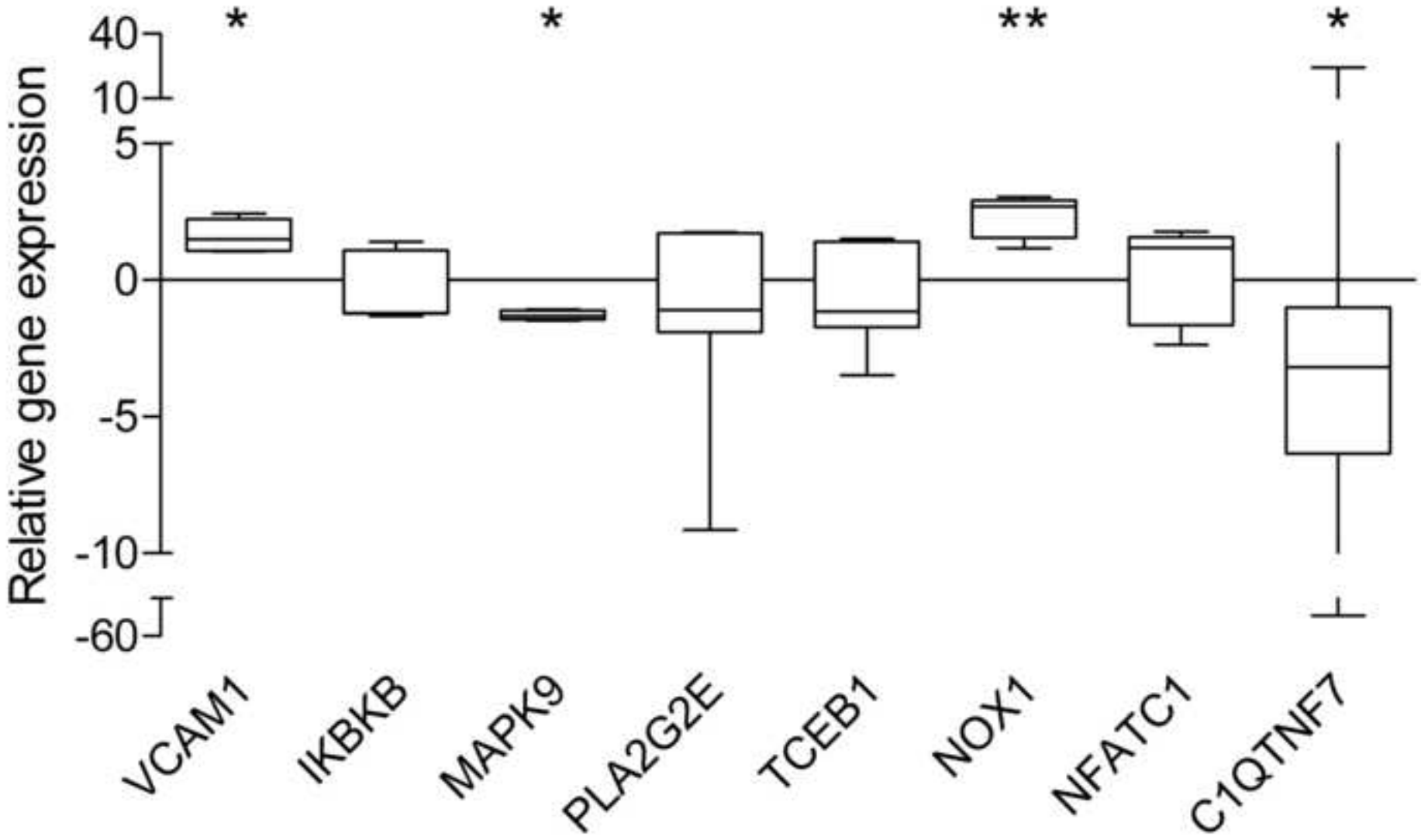

Figure 3

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