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Transcriptomics Data Integration Reveals Jak-STAT as a Common Pathway Affected by Pathogenic Intracellular Bacteria in Natural Reservoir Hosts

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Abstract

The study of the host-pathogen interface in natural reservoir hosts is essential to identify host-cell mechanisms affected by bacterial infection and persistence. Herein we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) to integrate transcriptomics data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium* during infection and persistence in two natural reservoir hosts, wild boar and sheep. The results showed that the upregulation of host innate immune pro-inflammatory genes and signaling pathways constitutes a general antibacterial mechanism in response to intracellular bacteria. Pathway focused analysis revealed a role for the Jak-STAT pathway during bacterial intracellular infection, a fact reported before in *Mycobacterium* infected cells but not during *Brucella* spp. and *A. phagocytophilum* infection. A clear activation of the Jak-STAT pathway was observed in *A. phagocytophilum* infected wild boar and sheep when compared to uninfected controls. *Brucella* spp. infection resulted in a balanced regulation of the Jak-STAT signaling and *M. bovis* infection of wild boar clearly produced a downregulation of some of the Jak-STAT effectors such as IL5 and TKY2. These results suggested that mycobacteria and brucellae induce host innate immune responses while manipulating adaptive immunity to circumvent host-cell defenses and establish infection. In contrast, *A. phagocytophilum* infection induces both innate and adaptive immunity, those suggesting that this pathogen uses other mechanisms to circumvent host-cell defenses by downregulating other adaptive immune genes and delaying the apoptotic death of neutrophils through activation of the Jak-STAT pathway among other mechanisms.

Keywords: Transcriptomics; Anaplasma; Mycobacterium; Brucella; Systems biology

Introduction

Pathogenic bacteria have to interact with host cells and reprogram the complex molecular and cellular networks of these cells to allow bacterial infection, replication and spread, while countering host-defense mechanisms [1,2]. This process is likely to involve genes from both pathogens and hosts, all of which are probably subject to complex regulation [1-3].

Molecular biology and in particular recent advances in genomics, transcriptomics and proteomics have allowed the characterization of host-pathogen interactions [2,3]. However, these studies have focused on the response of particular hosts to one or multiple pathogens, mostly using in vitro systems (see for example, [2]). Moving from in vitro studies in cultured cells to relevant animal disease models and natural reservoir hosts is crucial for understanding host-pathogen interactions, yet such studies are often neglected because cell culture-based systems are easier to manipulate. However, the study of the host-pathogen interface in natural reservoir hosts infected with different pathogens is now possible and essential to identify host-cell mechanisms affected by bacterial infection and persistence, which may be different from those identified in vitro [3,4].

Herein, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to integrate transcriptomics data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium* during infection and persistence in two natural reservoir hosts, wild boar (*Sus scrofa*) and sheep (*Ovis aries*).

Materials and Methods

Transcriptomics data

Transcriptomics data was obtained from previously published studies on infected and uninfected matching control animals using microarray hybridization and real-time RT-PCR in wild boar infected with *Mycobacterium bovis*, *Anaplasma phagocytophilum* and *Brucella suis* [4-6] (NCBI Gene Expression Omnibus (GEO) platform accession and series numbers GPL3533, GPL3533, GSE15766, GSE17492) and in sheep infected with *A. phagocytophilum* and *Brucella ovis* [7-9] (GPL4456, GPL6954, GSE11928 and GSE10286). In these studies, the infection with *M. bovis*, *A. phagocytophilum*, *B. ovis* or *B. suis* strains was characterized in experimentally or naturally infected animals during acute or chronic infection (Table 1).

Transcriptomics data integration and analysis

Microarray data from all host-pathogen interactions were filtered to select significant ($P < 0.05$) differentially expressed genes with an infected/uninfected fold change (FC) ≥ 1.2. These genes were analyzed using DAVID V6.7 (http://david.abcc.ncifcrf.gov/) [10,11] to select

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the highest enrichment score (ES), which is the geometric mean of all enrichment P values (EASE scores) for each gene ontology (GO) term [11] and clustering for host cell biological processes, molecular functions and pathways affected by bacterial infection in these hosts and then identify among them those factors common to all host-bacteria interactions (Figure 1). The significance of GO term enrichment was determined with a modified Fisher's exact test (EASE score; $P \leq 0.001$) and a FC > 2 for overrepresented terms. Enrichment P-values were globally corrected to control family-wide false discovery rates at Benjamini ≤ 0.0004. ES > 2 was used to rank GO term enrichment.

Real-time reverse transcription (RT)-PCR

Differential expression of genes in common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria was analyzed by real-time RT-PCR using primers designed based on sequences available in the GenBank (Table 2). The real-time RT-PCR was performed on pooled RNA samples from infected and uninfected wild boar and sheep (wild boar infected with *A. phagocytophilum*, N=2; wild boar infected with *Brucella* spp., N=3; wild boar infected with *M. bovis*, N=6; sheep infected with *A. phagocytophilum*, N=2; sheep infected with *Brucella* spp, N=6; wild boar uninfected controls, N=12; sheep uninfected controls, N=5) with gene specific primers using the iScript One-Step RT-PCR Kit with SYBR Green and the iQ5 thermal cycler (Bio-Rad, Hercules, CA, USA) following manufacturer's recommendations. The mRNA levels were normalized against cyclophlilyn and beta-actin using the genNorm method (ddCT method as implemented by Bio-Rad iQ5 Standard Edition, Version 2.0) [12]. In all cases, the mean of triplicate values was used and data from infected and uninfected animals were compared using the Student`s t-test (P=0.05). Correlation analysis between microarray and RT-PCR results was conducted in Excel by calculating (a) percent of values with similar tendency (i.e. no variation, upregulated or downregulated) and (b) correlation coefficients (R^2) between all values independently of the statistical analysis for RT-PCR results which were affected by the low number of samples used in the analysis.

Results and Discussion

The analysis conducted here focused on wild boar infected with *M. bovis*, *A. phagocytophilum* and *B. suis* [4-6] and sheep infected with *A. phagocytophilum* and *B. ovis* [7-9]. These pathogens represent intracellular bacteria that infect and replicate within host immune cells and were selected because of their impact as zoonotic pathogens in many regions of the world.

An analysis pipeline was developed using DAVID to integrate data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium* during infection and persistence in two natural reservoir hosts, Eurasian wild boar and sheep (Figure 1). Because transcriptomics data were obtained from different experiments with tissue samples collected at different infection times and conditions [4-9] (Table 1), differences between various host-pathogen interactions could be explained by different factors. These factors include differences in the transcriptomics methods employed (microarray and data analysis platforms), experimental conditions (natural or experimental infections), host tissues used for RNA extraction (peripheral blood mononuclear cells (PBMC) or spleen) and individual variability of both pathogens and hosts. However, we hypothesized that statistically significant common factors emerging despite all these differences, have a particular relevance in identifying host-pathogen interactions of different pathogenic intracellular bacteria in different hosts, thus allowing the identification of common mechanisms that may be used for infection characterization, control and prevention. Therefore, the analysis focused on common mechanisms affected by these bacteria in all host-pathogen interactions.

Common host-cell biological processes, molecular functions and pathways affected by *Anaplasma*, *Brucella* and *Mycobacterium* infection in wild boar and sheep

The results showed that it is possible to integrate data from different trascriptomics experiments to find common mechanisms affected by pathogenic intracellular bacteria in natural reservoir hosts. Common

Strain	Origin	Host	Tissue examined	Infection type	Characterization of infection
A. phagocytophilum	Isolated from infected sheep in the Basque Country, Spain [30] (Genbank accession number EU436164)	Sheep	PBMC	Experimental acute infection	Infection was confirmed by microscopic examination of stained blood smears and *msp4* PCR [7,30]
	Isolated from infected Eurasian wild boar hunter-killed in Slovenia, genotipically identical to strains isolated from humans, dogs and *I. ricinus* ticks [31] (Genbank accession numbers AY055469, AF033101 and EU246961)	Wild boar	PBMC	Natural chronic infection	Infection was confirmed by 16S rDNA and *groESL* PCRs and sequence analysis [31]
B. ovis R virulent PA strain	Provided by Dr. J.M. Verger. Unite´ d'Infectiologie Animale et Sante´ Publique, INRA, Nouzilly, France [32,33]	Sheep	PBMC	Experimental acute infection	Infection was confirmed at necropsy by bacterial culture, morphology, Gram staining, oxidase and urease tests, CO_2 requirements and phage typing [8,34]
B. suis biovar 2	Isolated from infected Eurasian wild boar in Navarra, Spain [34,35]	Wild boar	Spleen	Natural chronic infection	Infection was confirmed by bacterial culture and seroconversion [5,36]
M. bovis	Isolated from infected Eurasian wild boar in Southwestern Spain [3]	Wild boar	Spleen	Natural chronic infection	Infection was confirmed at necropsy by pathology, bacterial culture and spoligotyping [3,4]

Table 1: Bacterial strains and experimental animals.

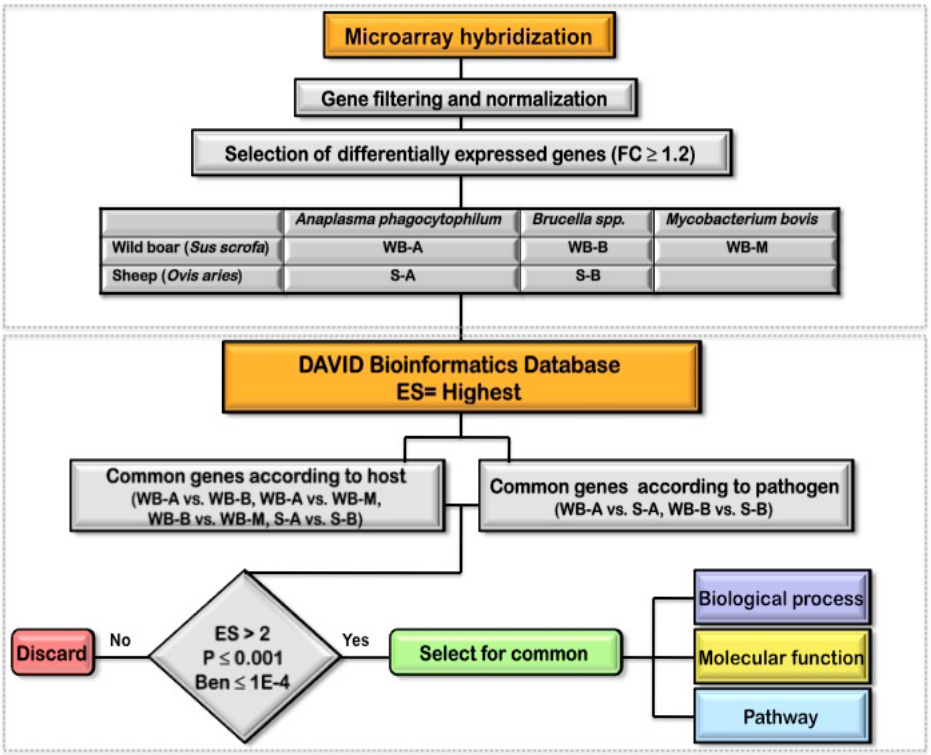

Figure 1: Analysis pipeline. The analysis used the Database for Annotation, Visualization and Integrated Discovery (DAVID) V 6.7 (http://david.abcc.ncifcrf.gov/) to integrate data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium* during infection in natural reservoir hosts, wild boar and sheep. Abbreviations: ES, enrichment score; FC, fold change; Ben, Benjamini; WB-A, wild boar infected with *A. phagocytophilum*; WB-B, wild boar infected with *B. suis*; WB-M, wild boar infected with *M. bovis*; S-A, sheep infected with *A. phagocytophilum*; S-B, sheep infected with *B. ovis*.

host-cell biological processes affected by *Anaplasma*, *Brucella* and *Mycobacterium* infection in wild boar and sheep included regulation of immune system and immune system with 33 genes represented (Tables 3 and 4). The common host-cell molecular functions affected included 28 genes with receptor binding, cytokine activity and growth factor activity (Tables 3 and 4). The common host-cell pathways affected by these bacteria were cytokine-receptor interaction, hematopoietic cell lineage and Janus Kinase-Signal Transducer and Activator of Transcription (Jak-STAT) signaling pathway (Table 3). A good correlation was obtained between microarray and RT-PCR results for genes in common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria (Table 4). Correlation between microarray and RT-PCR results was 0.36 (R^2=0.74), 0.55 (R^2=0.78) and 0.77 (R^2=0.81) for wild boar infected with *A. phagocytophilum*, *B. suis* and *M. bovis*, respectively, and 0.64 (R^2=0.79) and 0.70 (R^2=0.80) for sheep infected with *A. phagocytophilum* and *B. ovis*, respectively.

Effect of *Anaplasma*, *Brucella* and *Mycobacterium* infection on wild boar and sheep innate and adaptive immunity

These results showed that *Anaplasma*, *Brucella* and *Mycobacterium* infection of wild boar and sheep affect the expression of genes involved in host innate and adaptive immunity. However, not surprisingly, the way in which host immune response was affected varied between different host-bacteria interactions. Differences in host immune response between different host-pathogen interactions could be related to host/pathogen-specific factors and/or differences in gene expression between early (acute) and late (chronic) infections. Nevertheless, common to all bacteria-host interactions was the induction of innate immunity through upregulation of pro-inflammatory cytokines such as interleukins IL1B and/or IL6 that are induced in phagocytes after toll-like receptor (TLR) recognition resulting in activation of the complement system and pathogen opsonization for phagocytosis by macrophages and neutrophils [13]. As in previous experiments with cultured human macrophages infected with Gram-positive bacteria, Gram-negative bacteria and *M. tuberculosis* [2], shared responses included genes encoding receptors and signal transduction molecules affecting the cytokine-receptor interaction, hematopoietic cell lineage and Jak-STAT signaling pathways. However, adaptive immunity was induced through upregulation of genes such as cluster differentiation 4 (CD4) and IL21 only in wild boar and sheep infected with *A. phagocytophilum*.

The results obtained herein showed that the upregulation of host innate immune pro-inflammatory genes and signaling pathways constitute a general antibacterial mechanism in response to pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium*, a finding previously suggested in other studies with *Brucella* spp. [5,8,14], *Mycobacterium* spp. [2,4,15-20] and *A. phagocytophilum* [7,21].

Role for the Jak-STAT pathway during *Anaplasma*, *Brucella* and *Mycobacterium* infection of wild boar and sheep

Pathway-focused analysis revealed a role for the Jak-STAT pathway during bacterial intracellular infection, a fact reported before in

GenBank accession number[1]	Gene symbol	Upstream/downstream primer sequences (5´-3´)	
		Wild boar (*Sus scrofa*)	Sheep/Cattle (*Ovis aries/Bos taurus*)
NM_213844.2/ NM_001144097.1	CRP	**Ss-CRPF**: GTGTTGTCACCGGAGGAGAT **Ss-CRPR**: CCAGAGACAAGGGGAACGTA	**Oa-CRPF:** AGCATGTCCCGTACCAAAAG **Oa-CRPR:** TTTTGCCTTGACAGTTGCAG
NM_214155.2/ NM_001009417.1	CD247	**Ss-CD247F:** TGGGGAAGGACAAGATGAAG **Ss-CD247R:** TCTCTCAGGAACAGGGCAGT	**Bt-CD247F:** TTGTCACTGCCCTGTTTCTG **Bt-CD247R:** ACTTCGTGGGGGTTCTTCTT
NM_213775.2/ NM_001009382.1	CD3D	**Ss-CD3DF:** TCTCTCAGGAACAGGGCAGT **Ss-CD3DR:** AGGGAAGCGAAGAAAGAAGG	**Oa-CD3DF:** TTGAGGACCCAAGAGGAATG **Oa-CD3DR:** GTCTCATGTCCAGCAAAGCA
NM_001001908.1/ NM_001129902.1	CD4	**Ss-CD4F:** GCTGGGGAACCAGAGTATGA **Ss-CDFR:** AGAACCCAGCGAGAAACAGA	**Oa-CD4F:** AAGCTCGAGGTGGAACTGAA **Oa-CD4R:** CGTCCAGGTACCACTGTCCT
NM_213774.1/ NM_001034735.1	CD74	**Ss-CD74F:** CCTGCTCCTGAAGTCTGACC **Ss-CD74R:** GTGTCTCCTCCAGCGAGTTC	**Bt-CD74F:** TTGAGGGTCCACCAAAAGAC **Bt-CD74R:** GCTGATGGAGAGGCAGAGTC
NM_214269.2/ NM_174375.2	KITLG	**Ss-KITLGF:** GATGCCTTCAAGGATTTGGA **Ss-KITLGR:** ATGGAATCTGAGGCCTTCCT	**Bt-KITLGF:** CGTCCACACTCAAGGGATCT **Bt-KITLGR:** TTCCACCATCTCGCTTATCC
NM_214354.1/ NM_001076269.1	CALCR	**Ss-CALCRF:** TGGAATCTCCAATCCAGGAG **Ss-CALCRR:** AGCACCAGCGTGTAAGTGTG	**Bt-CALCRF:** CCCATCCTGAGAGCAACATT **Bt-CALCRR**: AACACGCATGAAAATCACCA
XM_001924460.1/ NM_001100293.1	CCR4	**Ss-CCR4F:** TCACAGGAATGGCCTTTTTC **Ss-CCR4R:** GACTGCTTGTTGGCTTCCTC	**Bt-CCR4F:** TGTTCACTGCTGCCTCAATC **Bt-CCR4R:** TAAGATGAGCTGGGGGTGTC
NM_001009580.1/ NM_001113174.1	CXCL12	**Ss-CXCL12F:** CAGTGTCCCCAGTGTGTCAG **Ss-CXCL12R:** CTCTCAAAGAATCGGCAAGG	**Bt-CCXCL12F:** GAGATCATGTCTCCGCCTTC **Bt-CCXCL12R:** GAAACTGTGCTGTGGCTTCA
U61139.1/ L07939.1	CSF2	**Ss-CSF2F:** TTACCATCCCCTTTGACTGC **Ss-CSF2R:** AGTCTGTGCCCCATTACAGC	**Oa-CSFF:** CGTCCAGGTACCACTGTCCT **Oa-CSFR:** GTTGGTCTAGGCAGCTCGTC
NM_001003924 / NM_001014945	C1QA	**Ss-C1QAF:** CTTCCAGGTGGTGTCCAAGT **Ss-C1QAR:** TGGATCCAGACCTTGTCTCC	**Bt-C1QAF:** GCATCTTCAGTGGCTTCCTC **Bt-C1QAR:** ACTTGGTAGGGCAGAGCAGA
AY349420.1/ NM_001046599	C1qB	**Ss-C1QBF:** GCGAGTCCGGAGACTACAAG **Ss-C1QBR:** ATGAGGTTCACGCACAGGTT	**Bt-C1QBF:** CTGCGACTACGTCCAGAACA **Bt-C1QBR:** GTTGGTGTTGGGGAGAAAGA
NM_001001646.1/ NM_001166616.1	C5	**Ss-C5F:** GCATGTCCCAGACCAAACTT **Ss-C5R:** ACGGCTTCTCCAGCTTTGTA	**Bt-C5F:** TGCTGAGAGAGACGCTGAAA **Bt-C5R:** TCAATCCAGGTCGAGGAATC
NM_214282.1/ NM_001045966.1	C7	**SsC7F:** TCAAGTGCCTCCTCTCCTGT **SsC7R:** GCTGATGCACTGACCTGAAA	**Bt-C7F:** GGCGGTCAATTGCTGTTTAT **Bt-CTR:** GGTCTGCTTTCTGCATCCTC
NM_213975.1/ NM_001009786.1	FTH1	**Ss-FTH1F:** TGCTTCAACAGTGCTTGGAC **Ss-FTH1R:** TCTTCAAAGCCACATCATCG	**Oa-FTH1F:** CGCTACTGGAACTGCACAAA **Oa-FTH1R:** CAGGGTGTGCTTGTCAAAGA
NM_001004027.1/ NM_001014912.1	HMOX1	**Ss-HMOX1F:** ATGTGAATGCAACCCTGTGA **Ss-HMOX1R:** GTGCTCTTGGTTGGGAAAGA	**Bt-HMOX1F:** ACTCACCCCTTCCTGTTCCT **Bt-HMOX1R:** CACAAAGCTGCTCCAACAAA
NM_001123124.1/ NM_174339.3	HIF1A	**Ss-HIF1AF:** TTACAGCAGCCAGATGATCG **Ss-HIF1AR:** TGGTCAGCTGTGGTAATCCA	**Bt-HIF1AF:** TCAGCTATTTGCGTGTGAGG **Bt.HIF1AR:** TCGTGGTCACATGGATGAGT
NM_214055.1/ NM_001009465.2	IL1B	**Ss-IL1BF:** CAGCCATGGCCATAGTACCT **Ss-IL1BR:** CCACGATGACAGACACCATC	**Oa-IL1BF:** CGAACATGTCTTCCGTGATG **Oa-IL1BR:** GAAGCTCATGCAGAACACCA
AY552750.1 / NM_001009734.1	IL15	**Ss-IL15F:** TTGTCCTGTGTGTTCGGTGT **Ss-IL15R:** GCAAAGCCTTTTGAGTGAGC	**Oa-IL15F:** TTTGGGCTGTATCAGTGCAG **Oa-IL15R:** AATAACGCGTAGCTCGAGGA
NM_214415.1/ NM_198832.1	IL21	**Ss-IL21F:** CGGGGAACATGGAGAAAATA **Ss-IL21R:** CAGCAATTCAGGGTCCAAGT	**Bt-IL21F:** CGGGGAACATGGAGAGAATA **Bt-IL21R:** GGCAGAAATTCAGGATCCAA
BU946820.1/ NM_001195219.1	IL25	**Ss-IL25F:** CTCACCTGCGTGTCACCTT **Ss-IL25R:** AATATGGCATGGCCTACTCG	**Oa-IL25F:** GCCCCCTGGAGATATGAGTT **Oa-IL25R:** AGAAAACGGTCTGGTTGTGG
NM_214340.1/ NM_001075142.1	IL4R	**Ss-IL4RF:** CCCATCTGCCTATCCGACTA **Ss-IL4RR:** TGACAATGCTCTCCATCAGC	**Bt-IL4F:** CTGAGCCCAGAGTCAAGTCC **Bt-IL4R:** CAGCTGTGGGTCTGAGTCAA
NM_214205.1/ NM_001009783.1	IL5	**Ss-IL5F:** TGGCAGAGACCTTGACACTG **Ss-IL5R:** CCCTCGTGCAGTTTGATTCT	**Oa-IL5F:** AAAGGCAAACGCTGAACATT **Oa-IL5R:** CAGAGTTTGATGCGTGGAGA
M80258.1/ NM_001009392.1	IL6	**Ss-IL6F:** CACCAGGAACGAAAGAGAGC **Ss-IL6R:** GTTTTGTCCGGAGAGGTGAA	**Oa-IL6F:** TGGAGGAAAAAGATGGATGC **Oa-IL6R:** TGCATCTTCTCCAGCATGTC
NM_001166043.1/ EI184569.1	IL9	**Ss-IL9F:** TATGTCTGCCCATTCCTTCC **Ss-IL9R:** CATGGCTGTTCACAGGAAAA	**Oa-IL9F:** CACCACCACACTTTTGCATC **Oa-IL9R:** ACCCACCCAGAGAGGAATCT

NM_001077213.2/NM_001078655.1	MIF	**Ss-MIFF:** GAACCGTTCCTACAGCAAGC **SS-MIFR:** CCGAGAGCAAAGGAGTCTTG	**Oa-MIFF:** CTCCTCTCCGAGCTCACG **Oa-MIFR:** TGTAGATCCTGTCCGGGCTA
NM_001009578.1/ NM_001046477.1	MSN	**Ss-MSNF:** TGACCCCACACACTCCTACA **Ss-MSNR:** CCATAGTGGGCCATCTGTCT	**Bt-MSNF:** AAGGAGAGTGAGGCTGTGGA **Bt-MSNR:** CCCATTCTCATCCTGCTCAT
NM_214379.1/ NM_001100921.1	PPARG	**Ss-PPARGF:** GCCCTTCACCACTGTTGATT **Ss-PPARGR:** GAGTTGGAAGGCTCTTCGTG	**Oa-PPARGF:** CCCTGGCAAAGCATTTGTAT **Oa-PPARGR:** ACTGACACCCCTGGAAGATG
AF527990.2/ ES414801.1	PSME1	**Ss-PSME1F:** AAGAAGGGGGAAGATGAGGA **Ss-PSME1R:** CTTCTCCTGGACAGCCACTC	**Oa-PSMEF:** AAGCCAAGGTGGATGTGTTC **Oa-PSMER:** AGGCACTGGGATGTCCAAT
AF139837.1/ XM_002693929.1	RGS1	**Ss-RGS1F:** GAGTCCGATCTTTTGCATCG **Ss-RGS1R:** TGATTTTCTGGGCTTCATCA	**Bt-RGS1F:** GTGGTCTGAATCCCTGGAAA **Bt-RGS1R:** GATTCTCGAGTGCGGAAGTC
NM_001012299.1/ NM_174176.2	SCG2	**Ss-SCG2F:** CATGCGTTTCCCTCCTATGT **Ss-SCG2R:** TCTCACGCTTCTGGTTGTTG	**Bt-SCG2F:** ACTGGAGAGAAGCCAGTGGA **Bt-SCG2R:** TATGGAGGCTTTGGATTTGC
AB258452.1/ GQ175957.1	TLR8	**Ss-TLR8F:** TGTCATTGCAGAGTGCAACA **Ss-TLR8R:** GAGAAACGCCCCATCTGTAA	**Oa-TLR8F:** CCTTGCAGAGGCTAATGGAG **Oa-TLR8R:** CTCTGCCAAAACAAGCCTTC
L43124.1/ NM_174484.1	VCAM1	**Ss-VCAMF:** ATCCAAGCTGCTCCAAAAGA **Ss-VCAMR:** GGCCCTGTGGATGGTATATG	**Bt-VCAMF:** GAACCGACAGCTCCTTTCTG **BT-VCAMR:** TCCCTGACATCACAGGTCAA
NM_001031797.1/ NM_001123003.1	FADD	**Ss-FADDF:** AGTATCCCCGAAACCTGACC **Ss-FADDR:** CAGGAAATGAGGGACACAGG	**Oa-FADDF:** TGCAGATATTGCTTGGCTTG **Oa-FADDR:** CAGCATTCATCTCCCCAACT
NM_001014971.1	COL5A1	**Ss-COL5F:** GGAGATCGAGCAGATGAAGC **Ss-COL5R:** GCCCCTTCGGACTTCTTATC	Sequence not available for *O. aries* or *B. taurus*
U83916.1/ NM_001164714.1	CTGF	**Ss-CTGFF:** CATGGCCTAAAGCCAGAGAG **Ss-CTGFR:** TGGCACACGATTTTGAATGT	**Oa-CTGFF**: CCTGGTCCAGACCACAGAGT **Oa-CTGFR**: GCAGCCAGAGAGCTCAAACT
NM_001129953.1/ U47636.1	DMP1	**Ss-DMP1F:** CACTGAATCCGAAGAGCACA **Ss-DMP1R:** CCTGGATTGTGTGGTGTCAG	**Oa-DMP1F:** AGCCCAGAGTCCACTGAAGA **Oa-DMP1R:** GTTTGTTGTGGTACGCATCG
AJ577089.1/ NM_001009769.1	FGF2	**Ss-FGF2F:** AGCGACCCTCACATCAAACT **Ss-FGG2R:** TCGTTTCAGTGCCACATACC	**Oa-FGF2F:** GTGCAAACCGTTACCTTGCT **Oa-FGF2R:** ACTGCCCAGTTCGTTTCAGT
AF052657.1/ NM_001009235.1	FGF7	**Ss-FGF7F:** TTTGCTGAACCCAATTCCTC **Ss-FGF7R:** CAGGAACCCCCTTTTGATTT	**Oa-FGF7F:** ATGAACACCCGGAGCACTAT **Oa-FGF7R:** GGGCTGGAACAGTTCACATT
NM_001103212.1/ NM_176669.3	STC1	**Ss-STC1F:** GCTCTACTTTCCAGCGGATG **Ss-STC1R:** TCTTCGTCACATTCCAGCAG	**Bt-STC1F:** AGCTGAACGTGTGCAGTGTC **Bt-STC1R:** CGTCTGCAGGATGTGAAAGA
NM_214198.1/ AY656798.1	TGFB3	**Ss-TGFB3F:** GATGAGCACATAGCCAAGCA **Ss-TGFB3R:** AGGTGTGACACGGACAATGA	**Oa-TGFB3F:** AGCGGTATATCGATGGCAAG **Oa-TGFB3R:** ATTGGGCTGAAAGGTGTGAC
NM_001114670.1/NM_001191344.1	TKY2	**Ss-TKY2F:** ACTGCTATGACCCGACCAAC **Ss-TKY2R:** TGACTTCTCGCCTTGGTCTT	**Oa-FLT4F:** AGCTAGCCACTCCTGCCATA **Oa-FLT4R:** TCTGTGTCAGCATCCGTCTC
NM_214292.1/ AY029232.1	EPOR	**Ss-EPORF:** CTACCAGCTTGAGGGTGAGC **Ss-EPORR:** CCACTTCGTTGATGTGGATG	**Oa-EPORF:** GTTGGTCTAGGCAGCTCGTC **Oa-EPORR:** TACTCAAAGCTGGCAGCAGA
DQ450679.1/ XM_002692067.1	IL15RA	**Ss-IL15F:** TTGTCCTGTGTGTTCGGTGT **Ss-IL15R:** GCAAAGCCTTTTGAGTGAGC	**Bt-IL15RAF:** AGGCTCCGGAACACACATAC **Bt-IL15RAR:** CACACTCTCCATGCTCTCCA
AY008846/AJ865374.1	Cyclophilin	**SsCYCLOPHILINL:** AGCACTGGGGAGAAAGGATT **SsCYCLOPHILINR:** CTTGGCAGTGCAAATGAAAA	**Oa-CyclophBF:** CTTGGCTAGACGGCAAACAT **Oa-CyclophBR:** GCTTCTCCACCTCGATCTTG
DQ845171/ U39357	Beta-actin	**SusBetActin-L**: GACATCCGCAAGGACCTCTA **SusBetActin-R**: ACACGGAGTACTTGCGCTCT	**ACTOVI5**: CTCTTCCAGCCTTCCTTCCT **ACTOVI3**:GGGCAGTGATCTCTTTCTGC

[1]GenBank accession numbers are shown for wild boar/sheep-cattle sequences.

Table 2: Primer sets used for analysis of differential gene expression by real-time RT-PCR.

Mycobacterium-infected cells [22-24] but not during *Brucella* spp. and *A. phagocytophilum* infection. This result highlighted the importance of integrating data from different trascriptomics experiments to discover common host-cell mechanisms affected by pathogenic intracellular bacteria.

In mammals, the Jak-STAT pathway is the principal signaling mechanism for a wide array of cytokines and growth factors such as CSF2, IL15, IL21, IL4R, IL5, IL6, IL9, TKY2, EPOR, IL15RA shown here to be differentially expressed in infected animals [25]. Jak activation stimulates cell proliferation, differentiation, cell migration and apoptosis resulting in hematopoiesis and immune development among other processes [25]. Predictably, downregulation of the Jak-STAT pathway activity affect these processes but failure to properly regulate Jak signaling cause inflammation, erythrocytosis and leukemia among other diseases [25]. Herein, a clear activation of the Jak-STAT pathway was observed in *A. phagocytophilum*-infected wild boar and sheep when compared to uninfected controls (Table 4). For *Brucella* spp., infection resulted in the upregulation of some ligands and the

Term	Count[1]	P value[2]	Fold change[3]	Benjamini[4]
Biological process (ES[5]=9.68)				
Regulation of immune system	21	1.5E-14	9.7	2.2E-11
Immune system	30	2.1E-14	5.4	1.6E-11
Molecular function (ES=10.27)				
Receptor binding	28	6.4E-15	6.2	1.8E-12
Cytokine activity	12	3.4E-09	12.0	4.7E-07
Growth factor activity	11	7.3E-09	13.0	5.1E-07
Pathway (ES=3.70)				
Cytokine-cytokine receptor interaction	16	6.0E-08	5.5	3.8E-06
Hematopoietic cell lineage	9	3.1E-06	9.5	9.8E-05
Jak-STAT signaling pathway	10	3.4E-05	5.9	4.4E-04

[1]Indicates the number of genes involved in individual GO terms. [2]Defines the significance of a GO term enrichment with a modified Fisher's exact test (EASE score), denoting if the term is over or under represented (if P ≤ 0.05, then terms are overrepresented). [3]Statistical threshold for GO term selection (FC > 2). [4]To globally correct enrichment P-values to control family-wide false discovery rate at Benjamini ≤ 0.0004. [5]Enrichment score (ES) was used to rank overall importance (enrichment) of GO terms.

Table 3: Common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria.

Gene symbol	Gene description	Host-bacteria interaction				
		WB-A	WB-B	WB-M	S-A	S-B
CRP	C-reactive protein, pentraxin-related	1.6 (ns)	ns (ns)	-2.0 (-3.3 ± 0.01)	ns (ns)	ns (ns)
CD247	CD247 molecule	1.6 (ns)	-2.2 (ns)	ns (ns)	ns (ns)	ns (ns)
CD3D	CD3d molecule, delta (CD3-TCR complex)	ns (ns)	ns (ns)	2.1 (ns)	-2.2 (ns)	ns (ns)
CD4	CD4 molecule	1.4 (ns)	ns (ns)	ns (ns)	1.3 (ns)	ns (ns)
CD74	CD74 molecule, major histocompatibility complex.	ns (ns)	-4.1 (ns)	-3.3 (-5.3 ± 0.2)	ns (ns)	ns (ns)
KITLG	KIT ligand	3.2 (ns)	ns (ns)	ns (ns)	-1.2 (ns)	ns (ns)
CALCR	Calcitonin receptor	5.3 (ns)	3.7 (ns)	ns (ns)	ns (ns)	ns (ns)
CCR4	Chemokine (C-C motif) receptor 4	ns (1.9±0.02)	ns (ns)	-2.3 (-8.1±0.05)	-1.4 (ns)	3.0 (ns)
CXCL12	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	1.4 (ns)	-4.8 (ns)	-9.8 (-2.5±0.02)	-2.7 (-4.5±2E-3)	ns (-11.1±8E-6)
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	1.4 (ns)	ns (ns)	ns (ns)	1.6 (6.1±4E-4)	ns (-4.2±4.5-6)
C1QA	Complement component 1, q subcomponent, A chain	1.3 (ns)	-2.8 (ns)	ns (-3.7 ± 0.2)	ns (ns)	ns (ns)
C1qB	Complement component 1, q subcomponent, B chain	1.3 (ns)	-3.9 (ns)	-9.0 (ns)	ns (ns)	ns (ns)
C5	Complement component 5	1.5 (ns)	ns ns	ns ns	1.3 (8.0±4E-4)	ns (-10.0±2E-6)
C7	Complement component 7	2.5 (ns)	ns (ns)	ns (ns)	1.3 (ns)	ns (ns)
FTH1	Ferritin, heavy polypeptide 1	ns (ns)	-3.6 (ns)	-4.1 (-2.5±0.7)	ns (ns)	ns (ns)
HMOX1	Heme oxygenase (decycling) 1	ns (ns)	-2.5 (ns)	-1.7 (-2.5±0.1)	ns (ns)	ns (ns)
HIF1A	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	1.3 (ns)	-2.0 (-3.0±0.02)	ns (ns)	ns (ns)	ns (ns)
IL1B	Interleukin 1, Beta	ns (ns)	2.9 (ns)	ns (ns)	1.3 (2.3±3E-4)	2.1 (1.4±5E-5)
IL15	Interleukin 15	ns (ns)	ns (ns)	ns (ns)	1.2 (ns)	2.7 (ns)
IL21	Interleukin 21	1.3 (ns)	ns (ns)	ns (ns)	1.2 (ns)	ns (ns)
IL25	Interleukin 25	ns (ns)	ns (ns)	ns (ns)	1.3 (ns)	1.6 (ns)
IL4R	Interleukin 4 receptor	1.2 (ns)	ns (ns)	ns (ns)	-2.0 (ns)	ns (ns)
IL5	Interleukin 5 (colony-stimulating factor, eosinophil)	1.3 (ns)	ns (10.8 ± 0.5)	ns (-3.1±3E-4)	ns (ns)	2.6 (ns)
IL6	Interleukin 6 (interferon, beta 2)	2.0 (ns)	ns (ns)	ns (ns)	1.2 (ns)	ns (1.1±4E-6)
IL9	Interleukin 9	ns (ns)	ns (ns)	ns (ns)	1.3 (ns)	1.3 (ns)
MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	ns (ns)	ns (ns)	ns (ns)	-2.0 (ns)	10.4 (ns)
MSN	Moesin	ns (ns)	-2.6 (ns)	-3.4 (ns)	ns (ns)	ns (ns)
PPARG	Peroxisome proliferator-activated receptor gamma	1.2 (ns)	-3.5 (ns)	ns (ns)	ns (ns)	ns (ns)

PSME1	Proteasome (prosome, macropain) activator subunit 1	ns (3.4 ± 2)	-3.1 (ns)	-2.4 (-3.5 ± 2.8)	ns (ns)	ns (ns)
RGS1	Regulator of G-protein signaling 1	ns (ns)	-2.0 (ns)	-1.9 (ns)	ns (ns)	ns (ns)
SCG2	Secretogranin II (chromogranin C)	1.6 ± (ns)	2.1 (ns)	ns (ns)	ns (ns)	ns (ns)
TLR8	Toll-like receptor 8	ns (ns)	-2.4 (ns)	ns (-2.72 ± 0.2)	1.2 (ns)	ns (ns)
VCAM1	Vascular cell adhesion molecule 1	1.6 (-3.2±0.02)	-4.8 (ns)	ns (-2.5± 0.2)	ns (ns)	ns (ns)
FADD	Fas (TNFRSF6)-associated via death domain	1.2 (ns)	ns (ns)	-10.8 (-2.8±0.6)	ns (ns)	ns (ns)
COL5A1	Collagen, type V, alpha 1	1.4 (ns)	-2.5 (ns)	ns (ns)	ns (ns)	ns (ns)
CTGF	Connective tissue growth factor	1.3 (1.3±0.3)	-2.8 (ns)	ns (ns)	ns (ns)	ns (ns)
DMP1	Dentin matrix acidic phosphoprotein 1	1.2 (ns)	1.9 (3.6±0.05)	ns (ns)	ns (ns)	ns (ns)
FGF2	Fibroblast growth factor 2 (basic)	1.6 (ns)	ns (ns)	ns (-3.1±2E-3)	1.3 (ns)	1.6 (ns)
FGF7	Hypothetical fibroblast growth factor 7 (keratinocyte growth factor)	1.4 (ns)	ns (ns)	ns (-2.6±0.1)	1.2 (1.9±7E-5)	ns (1.9±2E-5)
STC1	Stanniocalcin 1	1.4 (ns)	2.2 (12.1±0.01)	ns (ns)	ns (ns)	ns (ns)
TGFB3	Transforming growth factor, beta 3	1.3 (7.3±3E-4)	ns (ns)	ns (ns)	ns (ns)	3.2 (1.8 ± 4E-6)
TKY2	Tyrosine kinase 2	ns (ns)	-1.9 (ns)	-2 (-2.4±0.03)	ns (ns)	ns (ns)
EPOR	Ethropoietin receptor	1.4 (ns)	ns (ns)	ns (ns)	1.8 (ns)	ns (ns)
IL15RA	Interleukin 15 receptor, alpha	ns (ns)	ns (ns)	ns (ns)	1.4 (ns)	-1.6 (ns)

Data shows significant (P<0.05) fold change in differential gene expression (positive and negative values for upregulated and downregulated genes in infected animals, respectively) obtained in the microarray analyses and by real-time RT-PCR (shown in parenthesis; average±SD). Abbreviations: ns, not significant differences in gene expression levels between infected and uninfected animals; WB-A, wild boar infected with *A. phagocytophilum*; WB-B, wild boar infected with *B. suis*; WB-M, wild boar infected with *M. bovis*; S-A, sheep infected with *A. phagocytophilum*; S-B, sheep infected with *B. ovis*

Table 4: Differential expression of genes in common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria.

downregulation of others that may result in a balanced regulation of the Jak-STAT signaling to prevent negative effects associated with improper regulation of this pathway (Table 4). As previously reported [22-24], *M. bovis* infection of wild boar clearly produced a downregulation of some of the Jak-STAT effectors such as IL5 and TKY2 (Table 4).

Conclusions

These results suggested that mycobacteria and brucellae induce host innate immune responses while manipulating adaptive immunity through Jak-STAT pathway and other mechanisms to circumvent host-cell defenses and establish infection. In contrast, *A. phagocytophilum* infection induces both innate and adaptive immunity, those suggesting that this pathogen uses other mechanisms to circumvent host-cell defenses. These mechanisms may include dowregulation of other adaptive immune genes such as IL2 and IL4 [7,26] and delaying the apoptotic death of neutrophils [7,21,27,28] through activation of the Jak-STAT pathway among other mechanisms.

These results improved our understanding of host-pathogen interactions by characterizing common host-cell mechanisms affected by pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium* in natural reservoir hosts and provided insights into mechanisms of pathogenesis that could be used as targets for therapeutic intervention and vaccine development. In fact, some of the cytokine-receptor interactions described here such as those involving IL4 and IL6 have already been used to characterize the immune response to parenteral and oral Bacillus Calmette-Guérin (BCG) vaccination to prevent *M. bovis* infection in wild boar [6,29] and the protective response to the *B. melitensis* Rev 1 vaccine in sheep for the control of *B. ovis* [9], respectively.

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