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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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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 cyclophilin 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 transcriptomics 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
<i>A. phagocytophilum</i>	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 <i>msp4</i> PCR [7,30]
	Isolated from infected Eurasian wild boar hunter-killed in Slovenia, genotypically identical to strains isolated from humans, dogs and <i>I. ricinus</i> ticks [31] (Genbank accession numbers AY055469, AF033101 and EU246961)	Wild boar	PBMC	Natural chronic infection	Infection was confirmed by 16S rDNA and <i>groESL</i> PCRs and sequence analysis [31]
<i>B. ovis</i> 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]
<i>B. suis</i> 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]
<i>M. bovis</i>	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.

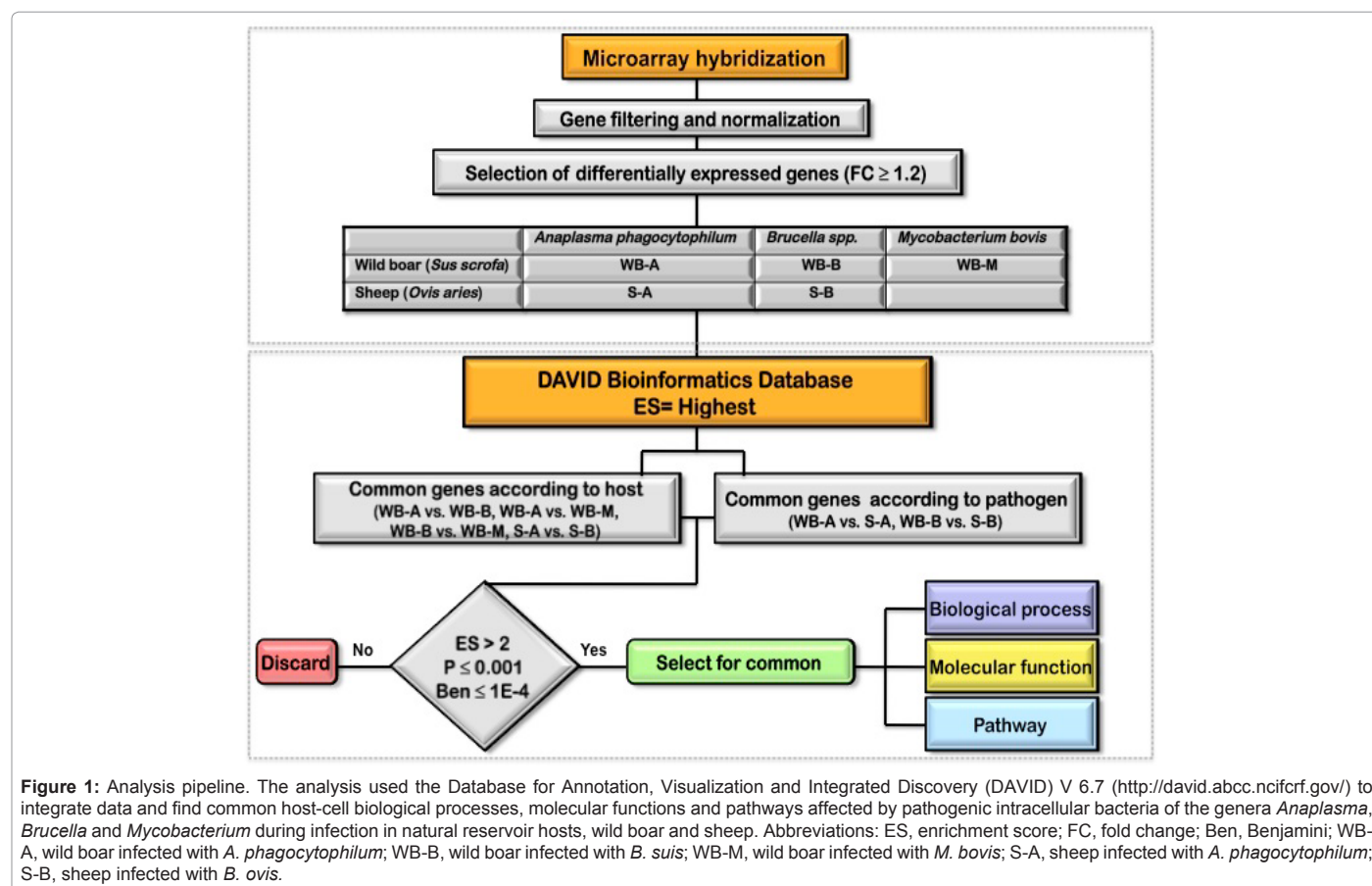


Figure 1: Analysis pipeline. The analysis used the Database for Annotation, Visualization and Integrated Discovery (DAVID) V 6.7 (<http://david.abcc.ncifcrf.gov/>) to integrate data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria 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 (<i>Sus scrofa</i>)	Sheep/Cattle (<i>Ovis aries/Bos taurus</i>)
NM_213844.2/ NM_001144097.1	CRP	Ss-CRPF: GTGTTGTCACCGGAGGAGAT Ss-CRPR: CCAGAGACAAGGGGAACGTA	Oa-CRPF: AGCATGTCCCGTACCAAAG Oa-CRPR: TTTTGCCTTGACAGTTGCAG
NM_214155.2/ NM_001009417.1	CD247	Ss-CD247F: TGGGGAAGGACAAGATGAAG Ss-CD247R: TCTCTCAGGAACAGGGCAGT	Bt-CD247F: TTGTCAGTGCCTGTTTCTG Bt-CD247R: ACTTCGTGGGGTTCTTCTT
NM_213775.2/ NM_001009382.1	CD3D	Ss-CD3DF: TCTCTCAGGAACAGGGCAGT Ss-CD3DR: AGGGAAGCGAAGAAAGAAGG	Oa-CD3DF: TTGAGGCCCAAGAGGAATG Oa-CD3DR: GTCTCATGTCCAGCAAAGCA
NM_001001908.1/ NM_001129902.1	CD4	Ss-CD4F: GCTGGGGAACAGAGTATGA Ss-CD4R: AGAACCCAGCGAAGAACAGA	Oa-CD4F: AAGCTCGAGGTGGAAGTGA Oa-CD4R: CGTCCAGGTACCAGTCTCT
NM_213774.1/ NM_001034735.1	CD74	Ss-CD74F: CCTGCTCCTGAAGTCTGACC Ss-CD74R: GTGTCTCCTCCAGCGAGTTC	Bt-CD74F: TTGAGGGTCCACAAAAGAC Bt-CD74R: GCTGATGGAGAGGCAGAGTC
NM_214269.2/ NM_174375.2	KITLG	Ss-KITLGF: GATGCCTTCAAGGATTTGGA Ss-KITLGR: ATGGAATCTGAGGCCCTTCT	Bt-KITLGF: CGTCCACACTCAAGGGATCT Bt-KITLGR: TTCCACCATCTCGTTATCC
NM_214354.1/ NM_001076269.1	CALCR	Ss-CALCRF: TGGAATCTCCAATCCAGGAG Ss-CALCRR: AGCACCAGCGTGAAGTGTG	Bt-CALCRF: CCCATCCTGAGAGCAACATT Bt-CALCRR: AACACGCATGAAAATCACCA
XM_001924460.1/ NM_001100293.1	CCR4	Ss-CCR4F: TCACAGGAATGGCCTTTTTC Ss-CCR4R: GACTGCTTGTGGCTTCTCTC	Bt-CCR4F: TGTTCAGTGCCTCAATC Bt-CCR4R: TAAGATGAGCTGGGGGTGTC
NM_001009580.1/ NM_001113174.1	CXCL12	Ss-CXCL12F: CAGTGTCCCAGTGTGTGTCAG Ss-CXCL12R: CTCTCAAAGAATCGGCAAGG	Bt-CXCL12F: GAGATCATGTCTCGCCTTC Bt-CXCL12R: GAAACTGTGCTGTGGCTTCA
U61139.1/ L07939.1	CSF2	Ss-CSF2F: TTACCATCCCCTTTGACTGC Ss-CSF2R: AGTCTGTGCCCATTAACAGC	Oa-CSF2F: CGTCCAGGTACCAGTCTCT Oa-CSF2R: GTTGGTCTAGGCAGCTCGTC
NM_001003924 / NM_001014945	C1QA	Ss-C1QAF: CTTCCAGGTGGTGTCCAAGT Ss-C1QAR: TGGATCCAGACCTGTCTCC	Bt-C1QAF: GCATCTTCAGTGGCTTCTCTC Bt-C1QAR: ACTTGGTAGGGCAGAGCAGA
AY349420.1/ NM_001046599	C1qB	Ss-C1QBF: GCGAGTCCGGAGACTACAAG Ss-C1QBR: ATGAGGTTCCAGCACAGGTT	Bt-C1QBF: CTGCGACTACGTCCAGAACA Bt-C1QBR: GTTGGTGTGGGGAGAAAGA
NM_001001646.1/ NM_001166616.1	C5	Ss-C5F: GCATGTCCCAGACCAAATT Ss-C5R: ACGGCTTCTCCAGCTTTGTA	Bt-C5F: TGCTGAGAGAGACGCTGAAA Bt-C5R: TCAATCCAGGTGAGGAATC
NM_214282.1/ NM_001045966.1	C7	Ss-C7F: TCAAGTGCCTCCTCTCTCTGT Ss-C7R: GCTGATGCACTGACCTGAAA	Bt-C7F: GGCGGTCAATTGCTGTTTAT Bt-C7R: GGTCTGCTTCTGCATCCTC
NM_213975.1/ NM_001009786.1	FTH1	Ss-FTH1F: TGCTTCAACAGTCTTGGAC Ss-FTH1R: TCTTCAAAGCCACATCATCG	Oa-FTH1F: CGTACTGGAAGTGCACAAA Oa-FTH1R: CAGGGTGTGCTTGTCAAAGA
NM_001004027.1/ NM_001014912.1	HMOX1	Ss-HMOX1F: ATGTGAATGCAACCTGTGA Ss-HMOX1R: GTGCTCTTGGTTGGGAAAGA	Bt-HMOX1F: ACTCACCCCTTCTGTTCTCT Bt-HMOX1R: CACAAAGTGTCTCAACAAA
NM_001123124.1/ NM_174339.3	HIF1A	Ss-HIF1AF: TTACAGCAGCCAGATGATCG Ss-HIF1AR: TGGTCACTGTGTAATCCA	Bt-HIF1AF: TCAGCTATTTGCGTGTGAGG Bt-HIF1AR: TCGTGGTACATGGATGAGT
NM_214055.1/ NM_001009465.2	IL1B	Ss-IL1BF: CAGCCATGGCCATAGTACCT Ss-IL1BR: CCACGATGACAGACACCATC	Oa-IL1BF: CGAATGTCTTCCGTGATG Oa-IL1BR: GAAGCTCATGCAGAACACCA
AY552750.1 / NM_001009734.1	IL15	Ss-IL15F: TTGCTCTGTGTGTTCCGGTGT Ss-IL15R: GCAAAGCCTTTTGTGAGTGC	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: CTCACCTGCGTGTACACCTT Ss-IL25R: AATATGGCATGGCCTACTCG	Oa-IL25F: GCCCCTGGAGATATGAGTT Oa-IL25R: AGAAAACGGTCTGGTTGTGG
NM_214340.1/ NM_001075142.1	IL4R	Ss-IL4RF: CCCATCTGCCTATCCGACTA Ss-IL4RR: TGACAATGCTCTCCATCAGC	Bt-IL4RF: CTGAGCCCAGAGTCAAGTCC Bt-IL4RR: CAGCTGTGGTCTGAGTCAA
NM_214205.1/ NM_001009783.1	IL5	Ss-IL5F: TGGCAGAGACCTTGACACTG Ss-IL5R: CCCTCGTGCAGTTTGATTCT	Oa-IL5F: AAAGGCAAACGCTGAACATT Oa-IL5R: CAGAGTTTGTGCGTGGAGA
M80258.1/ NM_001009392.1	IL6	Ss-IL6F: CACCAGGAACGAAAGAGAGC Ss-IL6R: GTTTTGTCCGGAGAGGTGAA	Oa-IL6F: TGGAGGAAAAAGATGGATGC Oa-IL6R: TGCATCTTCTCCAGCATGTC
NM_001166043.1/ E1184569.1	IL9	Ss-IL9F: TATGTCTGCCATTCCTTCC Ss-IL9R: CATGGCTGTTACAGGAAAA	Oa-IL9F: CACCACCACATTTTGCATC Oa-IL9R: ACCCACCAGAGAGGAATCT

NM_001077213.2/NM_001078655.1	MIF	Ss-MIFF: GAACCGTTCTACAGCAAGC SS-MIFR: CCGAGAGCAAAGGAGTCTTG	Oa-MIFF: CTCTCTCCGAGCTCACG Oa-MIFR: TGTAGATCCTGTCCGGGCTA
NM_001009578.1/ NM_001046477.1	MSN	Ss-MSNF: TGACCCACACACTCCTACA Ss-MSNR: CCATAGTGGGCCATCTGTCT	Bt-MSNF: AAGGAGAGTGAGGCTGTGGA Bt-MSNR: CCCATTCTCATCTGCTCAT
NM_214379.1/ NM_001100921.1	PPARG	Ss-PPARGF: GCCCTTACCACACTGTTGATT Ss-PPARGR: GAGTTGGAAGGCTCTTCGTG	Oa-PPARGF: CCCTGGCAAAGCATTGTGTAT Oa-PPARGR: ACTGACACCCCTGGAAGATG
AF527990.2/ ES414801.1	PSME1	Ss-PSME1F: AAGAAGGGGGAAGATGAGGA Ss-PSME1R: CTTCTCTGGACAGCCACTC	Oa-PSMEF: AAGCCAAGGTGGATGTGTTT Oa-PSMER: AGGCACTGGGATGTCCAAT
AF139837.1/ XM_002693929.1	RGS1	Ss-RGS1F: GAGTCCGATCTTTTGCATCG Ss-RGS1R: TGATTTTCTGGGCTTCATCA	Bt-RGS1F: GTGGTCTGAATCCCTGGAAA Bt-RGS1R: GATTCTCGAGTGCAGGAAGTC
NM_001012299.1/ NM_174176.2	SCG2	Ss-SCG2F: FATGCGTTTCCCTCCTATGT Ss-SCG2R: TCTCACGCTTCTGGTTGTTG	Bt-SCG2F: ACTGGAGAGAAGCCAGTGGAA Bt-SCG2R: TATGGAGGCTTTGGATTGTC
AB258452.1/ GQ175957.1	TLR8	Ss-TLR8F: TGTGATTGCAGAGTGCAACA Ss-TLR8R: GAGAAACGCCCATCTGTAA	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: TGAGATATTGCTTGGCTTG Oa-FADDR: CAGCATCTCCTCCCAACT
NM_001014971.1	COL5A1	Ss-COL5F: GGAGATCGAGCAGATGAAGC Ss-COL5R: GCCCTTCGGACTTCTTATC	Sequence not available for <i>O. aries</i> or <i>B. taurus</i>
U83916.1/ NM_001164714.1	CTGF	Ss-CTGF: CATGGCCTAAAGCCAGAGAG Ss-CTGFR: TGCCACACGATTTTGAATGT	Oa-CTGFF: CCTGGTCCAGACCAGAGAGT Oa-CTGFR: GCAGCCAGAGAGCTCAAAC
NM_001129953.1/ U47636.1	DMP1	Ss-DMP1F: CACTGAATCCGAAGAGCACA Ss-DMP1R: CCTGGATTGTGTGGTGTGAG	Oa-DMP1F: AGCCAGAGTCCACTGAAGA Oa-DMP1R: GTTTGTTGTGGTACGCATCG
AJ577089.1/ NM_001009769.1	FGF2	Ss-FGF2F: AGCGACCCTCACATCAAAC Ss-FGF2R: TCGTTTCAGTGCCACATACC	Oa-FGF2F: GTGCAAACCGTTACCTTGCT Oa-FGF2R: ACTGCCAGTTCGTTTCAGT
AF052657.1/ NM_001009235.1	FGF7	Ss-FGF7F: TTTGCTGAACCCATTCTC Ss-FGF7R: CAGGAACCCCTTTTGATTT	Oa-FGF7F: ATGAACACCCGGAGCACTAT Oa-FGF7R: GGGCTGGAACAGTTCACATT
NM_001103212.1/ NM_176669.3	STC1	Ss-STC1F: GCTCTACTTTCCAGCGGATG Ss-STC1R: TCTTCGTACATTCCAGCAG	Bt-STC1F: AGCTGAACGTGTGCAGTGTG Bt-STC1R: CGTCTGCAGGATGTGAAAGA
NM_214198.1/ AY656798.1	TGFB3	Ss-TGFB3F: GATGAGCACATAGCCAAGCA Ss-TGFB3R: AGGTGTGACACGGACAATGA	Oa-TGFB3F: AGCGGTATATCGATGGCAAG Oa-TGFB3R: ATTTGGCTGAAAGGTGTGAC
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: TTGTCTGTGTGTTCCGGTGT Ss-IL15R: GCAAAGCCTTTTGTGAGTGC	Bt-IL15RAF: AGGCTCCGGAACACACATAC Bt-IL15RAR: CACTCTCCATGCTCTCCA
AY008846/AJ865374.1	Cyclophilin	SsCYCLOPHILINL: AGCACTGGGGAGAAGGATT SsCYCLOPHILINR: CTTGGCAGTGCAAATGAAAA	Oa-CyclophBF: CTTGGCTAGACGGCAAACAT Oa-CyclophBR: GCTTCTCCACTCGATCTTG
DQ845171/ U39357	Beta-actin	SusBetActin-L: GACATCCGCAAGGACCTCTA SusBetActin-R: ACACGGAGTACTTGCGCTCT	ACTOVI5: CTCTCCAGCCTTCTTCTCT ACTOVI3: GGGCAGTGATCTCTTCTGTC

*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 transcriptomics 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 (ES⁵=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

¹Indicates the number of genes involved in individual GO terms. ²Defines 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 $P \leq 0.05$, then terms are overrepresented). ³Statistical threshold for GO term selection ($FC > 2$). ⁴To globally correct enrichment P-values to control family-wide false discovery rate at Benjamini ≤ 0.0004 . ⁵Enrichment 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 downregulation 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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References

- Bhavsar AP, Guttman JA, Finlay BB (2007) Manipulation of host-cell pathways by bacterial pathogens. *Nature* 449: 827-834.
- Nau GJ, Richmond JF, Schlesinger A, Jennings EG, Lander ES, et al. (2002) Human macrophage activation programs induced by bacterial pathogens. *Proc Natl Acad Sci USA* 5: 1503-1508.
- Naranjo V, Höfle U, Vicente J, Martín MP, Ruiz-Fons F, et al. (2006) Genes differentially expressed in oropharyngeal tonsils and mandibular lymph nodes of tuberculous and non-tuberculous European wild boars naturally exposed to *Mycobacterium bovis*. *FEMS Immunol Med Microbiol* 46: 298-312.
- Galindo RC, Ayoubi P, Naranjo V, Gortazar C, Kocan KM, et al. (2009) Gene expression profiles of European wild boar naturally infected with *Mycobacterium bovis*. *Vet Immunol Immunopathol* 129: 119-125.
- Galindo RC, Muñoz PM, de Miguel MJ, Marin CM, Labairu J, et al. (2010) Gene expression changes in spleens of the wildlife reservoir species, Eurasian wild boar (*Sus scrofa*), naturally infected with *Brucella suis* biovar 2. *J Gen Genomics* 37: 725-736.
- Lastra JM, Galindo RC, Gortázar C, Ruiz-Fons F, de la Fuente J, et al. (2009) Expression of immunoregulatory genes in peripheral blood mononuclear cells of European wild boar immunized with BCG. *Vet Microbiol* 134: 334-339.
- Galindo RC, Ayoubi P, García-Pérez AL, Naranjo V, Kocan KM, et al. (2008) Differential expression of inflammatory and immune response genes in sheep infected with *Anaplasma phagocytophilum*. *Vet Immunol Immunopathol* 126: 27-34.
- Galindo RC, Muñoz PM, de Miguel MJ, Marin CM, Blasco JM, et al. (2009) Differential expression of inflammatory and immune response genes in rams experimentally infected with a rough virulent strain of *Brucella ovis*. *Vet Immunol Immunopathol* 127: 295-303.
- Galindo RC, Muñoz PM, de Miguel MJ, Marin CM, Blasco JM, et al. (2009) Characterization of possible correlates of protective response against *Brucella ovis* infection in rams immunized with the *B. melitensis* Rev 1 vaccine. *Vaccine* 27: 3039-3044.
- Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, et al. (2003) DAVID:

- Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4: P3.
11. Huang DW, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nat Protoc* 4: 44-57.
 12. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408.
 13. Medzhitov R (2007) Recognition of microorganisms and activation of the immune response. *Nature* 449: 819-826.
 14. Eskra L, Mathison A, Splitter G (2003) Microarray analysis of mRNA levels from RAW264.7 macrophages infected with *Brucella abortus*. *Infect Immun* 71: 1125-1133.
 15. Goto Y, Nakamura RM, Takahashi H, Tokunaga T (1984) Genetic control of resistance to *Mycobacterium intracellulare* infection in mice. *Infect Immun* 46: 135-140.
 16. Ragno S, Romano M, Howell S, Pappin DJ, Jenner PJ, et al. (2001) Changes in gene expression in macrophages infected with *Mycobacterium tuberculosis*: a combined transcriptomic and proteomic approach. *Immunology* 104: 99-108.
 17. Keller C, Lauber J, Blumenthal A, Buer J, Ehlers S (2004) Resistance and susceptibility to tuberculosis analyzed at the transcriptome level: lessons from mouse macrophages. *Tuberculosis (Edinb)* 84: 144-158.
 18. Koul A, Herget T, Klebl B, Ullrich A (2004) Interplay between mycobacteria and host signalling pathways. *Nat Rev Microbiol* 2: 189-202.
 19. Naranjo V, Villar M, Martín-Hernando MP, Vidal D, Höfle U, et al. (2007) Proteomic and transcriptomic analyses of differential stress/inflammatory responses in mandibular lymph nodes and oropharyngeal tonsils of European wild boars naturally infected with *Mycobacterium bovis*. *Proteomics* 7: 220-231.
 20. Fernández de Mera IG, Pérez de la Lastra JM, Ayoubi P, Naranjo V, Kocan KM, et al. (2008) Differential expression of inflammatory and immune response genes in mesenteric lymph nodes of Iberian red deer (*Cervus elaphus hispanicus*) naturally infected with *Mycobacterium bovis*. *Dev Comp Immunol* 32: 85-91.
 21. Carlyon JA, Fikrig E (2003) Invasion and survival strategies of *Anaplasma phagocytophilum*. *Cell Microbiol* 5: 743-754.
 22. Jacobsen M, Reipsilber D, Kleinstueber K, Gutschmidt A, Schommer-Leitner S, et al. (2011) Suppressor of cytokine signaling-3 is affected in T-cells from tuberculosis TB patients. *Clin Microbiol Infect* 17: 1323-1331.
 23. Imai K, Kurita-Ochiai T, Ochiai K (2003) *Mycobacterium bovis* bacillus Calmette-Guérin infection promotes SOCS induction and inhibits IFN-gamma-stimulated JAK/STAT signaling in J774 macrophages. *FEMS Immunol Med Microbiol* 39: 173-180.
 24. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, et al. (2005) Hypervirulent *M. tuberculosis* W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *J Interferon Cytokine Res* 25: 694-701.
 25. Rawlings JS, Rosler KM, Harrison DA (2004) The JAK/STAT signaling pathway. *J Cell Sci* 117: 1281-1283.
 26. Kim HY, Mott J, Zhi N, Tajima T, Rikihisa Y (2002) Cytokine gene expression by peripheral blood leukocytes in horses experimentally infected with *Anaplasma phagocytophilum*. *Clin Diagn Lab Immunol* 9: 1079-1084.
 27. Borjesson DL, Kobayashi SD, Whitney AR, Voyich JM, Argue CM, et al. (2005) Insights into pathogen immune evasion mechanisms: *Anaplasma phagocytophilum* fails to induce an apoptosis differentiation program in human neutrophils. *J Immunol* 174: 6364-6372.
 28. Woldehiwet Z (2008) Immune evasion and immunosuppression by *Anaplasma phagocytophilum*, the causative agent of tick-borne fever of ruminants and human granulocytic anaplasmosis. *Vet J* 175: 37-44.
 29. Ballesteros C, Garrido JM, Vicente J, Romero B, Galindo RC, et al. (2009) First data on Eurasian wild boar response to oral immunization with BCG and challenge with a *Mycobacterium bovis* field strain. *Vaccine* 27: 6662-6668.

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