RESEARCH ARTICLE

**Nosema** Tolerant Honeybees (*Apis mellifera*) Escape Parasitic Manipulation of Apoptosis

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Abstract

Apoptosis is not only pivotal for development, but also for pathogen defence in multicellular organisms. Although numerous intracellular pathogens are known to interfere with the host’s apoptotic machinery to overcome this defence, its importance for host-parasite coevolution has been neglected. We conducted three inoculation experiments to investigate the apoptotic respond during infection with the intracellular gut pathogen *Nosema ceranae*, which is considered as potential global threat to the honeybee (*Apis mellifera*) and other bee pollinators, in sensitive and tolerant honeybees. To explore apoptotic processes in the gut epithelium, we visualised apoptotic cells using TUNEL assays and measured the relative expression levels of subset of candidate genes involved in the apoptotic machinery using qPCR. Our results suggest that *N. ceranae* reduces apoptosis in sensitive honeybees by enhancing inhibitor of apoptosis protein-2 (*iap*) gene transcription. Interestingly, this seems not be the case in *Nosema* tolerant honeybees. We propose that these tolerant honeybees are able to escape the manipulation of apoptosis by *N. ceranae*, which may have evolved a mechanism to regulate an anti-apoptotic gene as key adaptation for improved host invasion.

Introduction

In insects, epithelial cells of the intestine are typically the first line of pathogen defence. They produce not only antimicrobial peptides (AMPs) and reactive oxygen species (ROS) but they can also respond with programmed cell death (including apoptosis) of infected cells. The infection may then be simply cleared by defecation. Hence, it is not surprising to see that intracellular pathogens have evolved mechanisms to overcome apoptosis for self-protection and increase of reproductive success within their host cell [1–3]. This is also the case for microsporidia [4–6], a group of highly specialised intracellular fungal parasites, causing diseases in a wide range of animal hosts, including humans and several animal species important for agriculture and aquaculture [7].
Although strategies of numerous pathogens have been studied in some detail [1–3], adaptations by the host to withstand the manipulation by these pathogens are neglected. The inhibition of apoptosis was recently shown to be pivotal for a successful infection of the microsporidian pathogen *Nosema ceranae* [5], which is considered to be a major threat to honeybees and wild bee pollinators [8–10]. Several honeybee transcriptome studies also indicated a link between *Nosema* spp. infections and apoptosis and epithelium renewal [11–13], supporting the idea that apoptosis might play a central role during *Nosema* infection. *Nosema* spores are transmitted via the faecal–oral route and germinate in the host midgut and enter epithelial cells, where they replicate and produce a new generation of spores after 4 days [9, 14].

Because Nosemosis can seriously impact colony health, Danish bee breeders successfully selected for *Nosema* resistant colonies, which appear to be the result of tolerance at the individual level [15, 16]. To examine the importance of the apoptotic defence system in the adaptation of these *Nosema* tolerant honeybees, we compared them with sensitive honeybees in three controlled inoculation experiments and screened for apoptotic processes in the honeybee midgut epithelium.

**Materials and Methods**

**Experimental inoculation**

One colony of *Nosema* tolerant honeybees from Aarhus (Denmark) was transported to Avignon (France), where one colony with *Nosema* sensitive honeybees was chosen for positive controls. We replicated three independent inoculation experiments in May 2013 following standard methods [17]. Briefly, newly emerged workers (< 24 h old) were collected from the brood frames. *Nosema* sensitive (SN) and tolerant (TN) honeybees were individually fed with 10^5 freshly extracted and purified *N. ceranae* spores in 2 μl sucrose solution. Uninfected controls of the sensitive (SC) and tolerant (TC) honeybee strain were only fed with 2 μl sucrose solution. Individuals that have not consumed the inoculum were discarded from the experiment. Twenty worker bees per honeybee strain were housed in sterile stainless steel cages (10×10×5.5 cm) with a piece of clean wax foundation in an incubator at 34 ± 1°C, 60% relative humidity and provided with 50% (w/v) sucrose solution ad libitum. Bees were sacrificed either on one or six days post infection (p.i.). Their midguts (ventriculi without rectum) were dissected and stored accordingly to the analysis. We confirmed the treatment success by estimating the number of *Nosema* spp. spores for a random subset of 5 midgut samples for each treatment group and replicate using a Fuchs–Rosenthal haemocytometer under a phase-contrast microscope (×400).

**Immunohistochemistry**

4% buffered formaldehyde (Süss) was used for fixation of three midguts per replicate for 24 h at 8°C, followed by paraffin embedding according to standard histological methods. The ratio of apoptosis was determined by TUNEL (Terminal deoxynucleotidyl transferase mediated X-dUTP nick endlabelling) assays (In Situ Cell Death Detection Kit, Roche) on 7 μm thick longitudinal sections according to the manufacturer’s manual. This method allows the detection of apoptotic cells at the early stage, for which selective internucleosomal DNA degradation is characteristic, by directly labelling of single–and double–stranded DNA nicks with the enzyme TdT (Terminal deoxynucleotidyl transferase) and fluorescein–dUTP. Prior to the TUNEL reactions we blocked endogenous peroxidase activity (Dual Endogenous Enzyme Block, Dako), followed by permeabilisation step using nucleases–free 10 μg μl⁻¹ proteinase K in 10 mM Tris/ HCl pH 7.5 for 20 min at room temperature and rinsed the samples twice in PBS (phosphate–buffered saline). The TUNEL reaction was stopped after 1 h at 37°C in the dark by rinsing the
samples three times in PBS and then counterstaining with 1 μg ml⁻¹ DAPI (4',6-diamidino-2-phenylindole) (Sigma-Aldrich). We visualised apoptotic cells (TUNEL+ve) relative to the total number of cells (DAPI+ve) in the posterior part of midguts (primary site of the infection on day 6 p.i.) using fluorescence microscopy and acquired images with CCD camera connected to Axio Vision 4.6 (Zeiss). Automatic cell counting and analyses were performed with ImageJ [18] screening at the average 325 ± 16 s.e. cells per sample (see also S2 Table).

**Gene expression**

Midguts of nine workers for each treatment group and for each replicated experiment were sampled in pools of three individuals, flash-frozen in liquid nitrogen and stored at -80°C until subsequent qPCR analyses. Briefly, total RNA was isolated using TRIZOL extraction procedure and 1 μg RNA each were reverse transcribed. Obtained cDNA were purified using QIAquick PCR Purification Kit (Qiagen). We performed a TBLASTN search [19] of *Apis mellifera* (taxid 7460) database using full-length amino acid sequences for key proteins involved in apoptosis known from *Drosophila melanogaster* [20]. Only homologous proteins with at least 20% identity were considered for this study (S1 Table). Thus some proteins known to be relevant for apoptosis in *D. melanogaster* were not included due to insufficient homology. Gene specific primers spanning one intron of subset of nine potential candidate genes, including the genes *basket* (*bsk*), *tumor protein p53-like* (*p53*), *inhibitor of apoptosis protein 2* (*iap–2; homologous gene to Diap–1*), *caspase-2-like* (*casp–2; possible homologous gene to Dronc*) and *caspase-10-like* (*casp–10; homologous gene to Dredd*), were designed using Primer-BLAST on the *A. mellifera* reference genome (release v.4.5, GenBank, S1 Table). *Ribosomal protein S5a* (*RpS5a*) and *actin related protein 1* (*arp1, also known as actin*) were initially chosen in order to standardize expression levels between pools and treatment groups [21]. For qPCR, we used 20 ng cDNA mixed with 5 μl SensiMixPlus (Bioline), 0.25 μM of each primer and DEPC-water in 10 μl final
volume. Initial denaturation at 95°C for 10 min was followed by 40 amplification cycles (95°C for 15 s, 59°C for 30 s, 72°C for 30 s), ending with melting curve analysis from 50°C to 98°C in 1°C increments. At least two technical replicates were run per sample using Chromo4™ (Bio-Rad) and repeated if necessary to obtain a delta Cₜ (threshold cycle) value below 0.5 between two replicates (LinRegPCR [22]). Correct amplicon sizes absence of non-specific products were verified using the high-resolution automatic capillary electrophoresis system QIAxcel™ (Qiagen). We tested the suitability of RpS5a and arp1 as previously described [23], and found the housekeeping gene RpS5a (s.d. = 0.86) to be more suitable for normalisation of gene expression levels among samples, and thus excluded the arp1 (s.d. = 1.55) from further analyses.

Statistics
All statistical analyses and data plotting (mean ± s.e.) were performed in R (v.3.0.2) [24]. Spore load between infected groups was tested using Welch’s two-tailed t-test. We used Generalized Linear Models (GLM) based on quasilikelihood estimation with a binomial error distribution to test the effects of honeybee strain and treatment and their interactions on the apoptosis ratio for each day p.i. independently. Post-hoc analyses were performed using the Tukey’s HSD. The effects of day p.i., honeybee strain and treatment and their interactions on relative gene expression were tested using linear models (LM) for each gene separately, accounting for multiple testing with Bonferroni adjustments. We used the likelihood ratio test to test single parameters and their interactions, comparing the goodness-of-fit between the models [25]. If a model was found to be unstable with all interactions included, we removed non-significant interactions step-wise. Model validity was tested by comparing full models to their null models without any fixed factors included using likelihood ratio test. Tukey’s HSD post-hoc contrast analyses were performed using the glht function with Bonferroni adjustment (multcomp package, v.1.3–2.).

Results and Discussion
There were generally higher apoptosis rates in all four treatment groups tested on day 1 p.i. than on day 6 p.i. (GLM: day, estimate ± s.e. = -0.904 ± 0.239, P < 0.001, Fig 1B), suggesting that midgut epithelial cells in those young bees were still undergoing morphogenetic developments at age of 1–2 day [26]. More interestingly, however, was the interaction between treatment and honeybee strain (GLM: -0.899 ± 0.293, P < 0.005; Fig 1B) whereby N. ceranae infection reduced the rate of apoptosis in sensitive honeybees (Tukey’s HSD: -0.679 ± 0.210, P < 0.007), confirming the suppression of apoptosis by microsporidia [5], but interestingly this was not the case in the infected tolerant honeybees (Tukey’s HSD: 0.220 ± 0.205, P = 1). Hence, these results suggest an adaptive mechanism in the tolerant honeybees which enables them to remove infected cells from the epithelia into the gut lumen and presumably to eventually defecate.

As honeybees usually defecate during short defecation flights and avoid defecation in their nest [27], infected apoptotic cells have presumably been accumulated in the midgut and rectum in Nosema tolerant honeybees at this early stage of the infection in our cage experiments. This in fact would be a plausible explanation why we have not counted less numbers of Nosema spores between sensitive (SN: 6.0 ± 1.2×10⁶ spores; n = 14) and tolerant honeybees (TN: 8.0 ± 1.4×10⁶ spores; n = 15) on day 6 p.i. (t-test: t = 1.115, d.f. = 26.77, P < 0.275).

To explore the molecular mechanisms underlying the inhibition of apoptosis in the sensitive honeybee, we measured relative gene expression levels of candidate genes in the apoptotic cascade predicted from Drosophila [20] (S1 Table). We found age-related alterations in gene expression levels for Jun N-terminal kinase (JNK)/basket (bsk), linear model (LM): day p.i., 0.091 ± 0.026, P < 0.007; Fig 2) and tumor suppressor protein p53 (p53, LM: day p.i., 0.022 ± 0.005, P < 0.001; Fig 2), which were slightly higher expressed on day 6 p.i. than on day
1 p.i. over all treatment groups. Although both JNK/bsk and p53 are important proapoptotic factors [28, 29], the core of the apoptotic–machine consists of caspases that destroy essential cell proteins that initiate apoptosis [20, 30, 31]. Surprisingly, caspase–10–like (homologous gene to Dredd in D. melanogaster) was slightly higher expressed in the sensitive honeybees regardless the treatment than in the tolerant honeybees on day 6 p.i. (casp–10, LM: honeybee strain, 0.286 ± 0.093, P < 0.025; Fig 2). The caspase–2–like gene (possible homologous gene to Dronc in D. melanogaster), however, was not differentially expressed between treatment groups (casp–2, LM: 0.074 ± 0.074, P < 0.290; Fig 2). In D. melanogaster the apical cell-death caspase DRONC, mediated by the adapter ARK (homolog to the apoptotic protease-activating factor 1, Apaf 1), plays a central role due to its chronic activation in many cells [20]. This may also be the case for casp–2 in honeybees as we measured relatively high expression levels in all treatment groups. Nevertheless, cells survive due to the DIAP1 expression, which suppresses DRONC and other activated caspases [20, 32]. Interestingly, we found an interaction between honeybee strain and treatment on day 6 p.i. (LM: honeybee strain × treatment: 0.751 ± 0.260, P < 0.038; Fig 2), whereby iap–2 (predicted homologous gene of Diap–1 in D. melanogaster) expression appeared to be tenfold increased on average in sensitive honeybees when infected with N. ceranae (Tukey’s HSD: 0.761 ± 0.198, P < 0.001) but we found no alterations in iap–2 expression level in tolerant honeybees (Tukey’s HSD: 0.010 ± 0.169, P < 0.980).

Although IAPs are also known to play a regulatory role in pathogen-sensing pathways and induction of the innate immune system [20, 32–34], we speculate that the up-regulation of iap–2 in Nosema infected sensitive honeybees might be rather involved in cell survival, because previous studies reported that N. ceranae causes immunosuppression in sensitive honeybees [16, 35, 36]. Furthermore, this up-regulation of iap–2 only in Nosema infected sensitive honeybees would also plausibly explain the reduced apoptosis activity in sensitive honeybees in our TUNEL assays.

Fig 2. Relative expression (mean ± s.e.) of candidate genes important for apoptosis in Nosema infected honeybees. Nosema sensitive (SN, solid circles) and tolerant (TN, solid squares) honeybees infected with 10^6 N. ceranae spores, and their controls uninfected (SC, open circles and TC, open squares), were sampled at 1 day (green) and 6 days (blue) after inoculation. The genes JNK/bsk (Jun N–terminal kinase/basket), p53 (tumor protein p53-like), iap–2 (inhibitor of apoptosis protein 2; predicted homologous gene to Diap–1 in D. melanogaster), casp–2 (caspase–2–like; homologous gene to Dcp–1), casp–10 (caspase–10–like; homologous gene to Dredd) were predicted from Drosophila melanogaster. Sample sizes are ranging between six and ten pools of three individual honeybee midguts (see also S3 Table). Significance between treatment groups ***, P < 0.001.

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and supports previous findings [5]. Unfortunately, we did not measure the expression levels of potential apoptosis inducing proteins such as Reaper, Hid and Grim (known as RHG proteins), which can negatively regulate DIAP1 activity in *D. melanogaster* [20, 34]. Nevertheless, *in vitro* studies have demonstrated in some more detail that infections with protozoans such as *Toxoplasma gondii* [37], *Cryptosporidium parvum* [38] as well as bacteria *Shigella flexneri* [39] and *Neisseria gonorrhoeae* [40] elicit up-regulation of *iap* genes and result in the inhibition of host cell apoptosis in mammalian host cell cultures. The activation of the key transcription factor NF-kB was shown to correlate with *iap* transcription [37, 40] and may also be the case in our *in situ* honeybee-*Nosema* system and might be triggered by elevated *Nosema* HSP70 levels [41, 42]. The capability to retain high apoptotic activity in spite of a *Nosema* infection might explain why the tolerant honeybees can overcome the infection and eliminate the disease from the colony [15, 16]. Workers presumably simply clear the infection by removing those apoptotic infected cells on defecation flights. In contrast sensitive honeybees might be likely to retain the infection in the gut epithelium for much longer time. The dynamics of intestine epithelium development [26] may also provide an alternative explanation for the age-dependent *Nosema* susceptibility in *Bombus terrestris* and honeybees previously exclusively attributed to age polyethism [8, 43].

Our results provide a snap shot of host-parasite co-evolution, where artificial selection of the honeybee host has presumably accelerated a counter adaptation towards *Nosema*. Irrespective of the actual molecular mechanisms, this study does not only highlight the central role of apoptosis for host immunity in general but also shows the importance of its manipulation for intracellular pathogens. Understanding the molecular dialogue between infecting pathogen and host cell might not only be interesting for evolutionary biology and parasitology, but may also provide novel perspectives for effective immunological strategies in the treatment of animal and human diseases by interfering into the regulatory machinery of apoptosis.

Supporting Information

S1 Table. Primer sequences used in qPCR.

(SDOCX)

S2 Table. Data of the estimation of the apoptosis rate in the posterior end of honeybee midguts for days one and six post infection (d.p.i).

(SDOCX)

S3 Table. Relative gene expression data of predicated candidate genes (*bsk*, *p53*, *iap−2*, *casp−2* and *casp−10*).

(SDOCX)

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Author Contributions

Conceived and designed the experiments: CK YLC PK RFAM. Performed the experiments: CK CD. Analyzed the data: CK SE OL TM MW RFAM. Contributed reagents/materials/analysis tools: RFAM YLC CK PK TM. Wrote the paper: CK RFAM.
References

1. Bruchhaus I, Roeder T, Rennenberg A, Heussler VT. Protozoan parasites: programmed cell death as a mechanism of parasitism. Trends Parasitol. 2007; 23(8):376–83. doi: 10.1016/j.pt.2007.06.004 PMID: 17598817

2. Faherty CS, Maurelli AT. Staying alive: bacterial inhibition of apoptosis during infection. Trends Microbiol. 2008; 16(4):173–80. doi: 10.1016/j.tim.2008.02.001 PMID: 18353648

3. Mocarski ES, Upton JW, Kaiser WJ. Viral infection and the evolution of caspase-regulated apoptotic and necrotic death pathways. Nat Rev Immunol. 2012; 12(2):79–88. doi: 10.1038/nri3131

4. Del Aguila C, Izquierdo F, Granja AG, Hurtado C, Fenoy S, Fresno M, et al. Encephalitozoon cuniculi modulates p53-mediated apoptosis in infected cells. Int J Parasitol. 2006; 36(8):869–76. doi: 10.1016/j.ijpara.2006.04.002 PMID: 16753166

5. Higes M, Juaranaz A, Dias-Almeida J, Lucena S, Botias C, Meana A, et al. Apoptosis in the pathogenesis of Nosema ceranae (Microsporidia: Nosematidae) in honey bees (Apis mellifera). Environ Microbiol Rep. 2013; 5(4):530–6. doi: 10.1111/1758-2229.12099 PMID: 23864567

6. Scanlon M, Leitch GJ, Shaw AP, Moura H, Visvesvara GS. Susceptibility to apoptosis is reduced in the microsporidia-infected host cell. J Eukaryot Microbiol. 1999; 46(5):34S–5S. PMID: 10519237

7. Keeling P. Five Questions about Microsporidia. PLoS Pathog. 2009; 5(9). doi: 10.1371/journal.ppat.1000489 PMID: 19779558

8. Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature. 2014; 506(7488):364–9. doi: 10.1038/nature12977 PMID: 24553241

9. Higes M, Juarranaz A, Dias-Almeida J, Lucena S, Botias C, Meana A, et al. Apoptosis in the pathogenesis of Nosema ceranae (Microsporidia: Nosematidae) in honey bees (Apis mellifera). Environ Microbiol Rep. 2013; 5(4):530–6. doi: 10.1111/1758-2229.12099 PMID: 23864567

10. Goulson D, Nicholls E, Botias C, Rotheray EL. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science. 2015; 347(6229):1435–8. doi: 10.1126/science.1255957

11. Aufauvre J, Misme-Aucouturier B, Vigues B, Texier C, Delbac F, Blot N. Transcriptome analyses of the honeybee response to Nosema ceranae and insecticides. PLoS ONE. 2014; 9(3). doi: 10.1371/journal.pone.0091686 PMID: 24646894

12. Dussaubat C, Brunet JL, Higes M, Colbourne JK, Lopez J, Choi JH, et al. Gut pathology and responses to the microsporidium Nosema ceranae in the honey bee Apis mellifera. PLoS ONE. 2012; 7(5). doi: 10.1371/journal.pone.0037017 PMID: 22623972

13. Holt HL, Aronstein KA, Grozinger CM. Chronic parasitization by Nosema microsporida causes global expression changes in core nutritional, metabolic and behavioral pathways in honey bee workers (Apis mellifera). BMC Genomics. 2013; 14: doi: 10.1186/1471-2164-14-799 PMID: 24245482

14. Gisdar S, Mockel N, Linde A, Genersch E. A cell culture model for Nosema ceranae and Nosema apis allows new insights into the life cycle of these important honey bee-pathogenic microsporidia. Environ Microbiol. 2011; 13(2):404–13. doi: 10.1111/j.1462-2920.2010.02346.x PMID: 20880328

15. Hatjina F, Bienkowska M, Charistos L, Chlebo R, Costa C, Dražić MM, et al. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. J Apicult Res. 2014; 53(3):337–63. doi: 10.3896/IBRA.1.53.3.02

16. Huang Q, Kryger P, Le Conte Y, Moritz RFA. Survival and immune response of drones of a Nosemosis tolerant honey bee strain towards N. ceranae infections. J Invertebr Pathol. 2012; 109(3):297–302. doi: 10.1016/j.jip.2012.01.004 PMID: 22285444

17. Fries I, Chauzat MP, Chen YP, Doublet V, Genersch E, Gisdar S, et al. Standard methods for Nosema research. J Apicult Res. 2013; 52(1). doi: 10.3896/ibra.1.52.1.14

18. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012; 9(7):671–5. doi: 10.1038/nmeth.2089 PMID: 22930834

19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215(3):403–10. doi: 10.1016/s0022-2836(05)80320-0.
23. Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper—Excel-based tool using pair-wise correlations. Biotechnol Lett. 2004; 26(6):509–15. doi: 10.1023/b:bile.0000019559.84305.47 PMID: 15127793

24. R Foundation for Statistical Computing R: a language and environment for statistical computing (25 September 2013).

25. Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed effects models and extensions in ecology with R: Springer; 2009.

26. Pipan N, Rakovec V. Cell death in the midgut epithelium of the worker honey bee (Apis mellifera carnica) during metamorphosis. Zoomorphologie. 1980; 94(2):217–24. doi: 10.1007/bf01081936

27. Seeley TD, Nowicke JW, Meselson M, Guillemin J, Akhratanakul P. Yellow Rain. Sci Am. 1985; 253(3):122–31.

28. Kanda H, Miura M. Regulatory roles of JNK in programmed cell death. J Biochem. 2004; 136(1):1–6. doi: 10.1093/jb/mvh098 PMID: 15269233

29. Steller H. Drosophila p53: meeting the Grim Reaper. Nat Cell Biol. 2000; 2(6):E100–E2. doi:10.1038/35014093 PMID: 10854336

30. Thornberry NA, Lazebnik Y. Caspases: Enemies within. Science. 1998; 281(5381):1312–6. doi:10.1126/science.281.5381.1312 PMID: 9721091

31. Hay BA, Guo M. Caspase-dependent cell death in Drosophila. Annu Rev Cell Dev Biol. 2006; 22:623–50. doi:10.1146/annurev.cellbio.21.012804.093845 PMID: 16842034

32. Molestina RE, Payne TM, Coppens I, Sinai AP. Activation of NF-kappa B by Toxoplasma gondii correlates with increased expression of antiapoptotic genes and localization of phosphorylated I kappa B to the parasitophorous vacuole membrane. J Cell Sci. 2003; 116(21):4359–71. doi:10.1242/jcs.006832

33. Liu J, Enomoto S, Lancto CA, Abrahamsen MS, Rutherford MS. Inhibition of apoptosis in Cryptosporidium parvum-infected intestinal epithelial cells is dependent on survivin. Infect Immun. 2008; 76(8):3784–92. doi:10.1128/iai.00308-08 PMID: 18919556

34. Pedron T, Thibault C, Sansonetti PJ. The invasive phenotype of Shigella flexneri directs a distinct gene expression pattern in the human intestinal epithelial cell line Caco-2. J Biol Chem. 2003; 278(36):33878–86. doi:10.1074/jbc.M303749200 PMID: 12813033

35. Binnicker MJ, Williams RD, Apicella MA. Infection of human urethral epithelium with Neisseria gonorrhoeae elicits an upregulation of host anti-apoptotic factors and protects cells from staurosporine-induced apoptosis. Cell Microbiol. 2003; 5(8):549–60. doi:10.1046/j.1462-5822.2003.00300.x PMID: 12864814

36. Vidau C, Panek J, Texier C, Biron DG, Belzunces LP, Le Gall M, et al. Differential proteomic analysis of midguts from Nosema ceranae-infected honeybees reveals manipulation of key host functions. J Invertebr Pathol. 2014; 121:89–96. doi:10.1016/j.jip.2014.07.002 PMID: 25038465

37. Joly A-L, Wettstein G, Mignot G, Ghiringhelli F, Garrido C. Dual Role of Heat Shock Proteins as Regulators of Apoptosis and Innate Immunity. J Innat Immun. 2010; 2(3):238–47. doi:10.1159/000296508

38. Smart MD, Sheppard WS. Nosema ceranae in age cohorts of the western honey bee (Apis mellifera). J Invertebr Pathol. 2012; 109(1):148–51. doi:10.1016/j.jip.2011.09.009 PMID: 22001631