Apparent effect of chronic *Plasmodium* infections on disease severity caused by experimental infections with *Mycoplasma gallisepticum* in house finches

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ABSTRACT

An epidemic caused by a successful host jump of the bacterial pathogen *Mycoplasma gallisepticum* from poultry to house finches in the 1990s has by now spread across most of North America. *M. gallisepticum* causes severe conjunctivitis in house finches. We experimentally show that *M. gallisepticum* transmission to birds with or without chronic *Plasmodium* infection does not differ. However, once infected with *M. gallisepticum* house finches chronically infected with *Plasmodium* develop more severe clinical disease than birds without such infection. We speculate as to possible effects of coinfection.

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1. Introduction

Chronic infections with *Plasmodium* may adversely impact birds as experimentally shown by effects on reproduction (Merino et al., 2000; Knowles et al., 2010), on feather growth and quality (Marzal et al., 2013) or on telomere-length degradation (Asghar et al., 2015, 2016). *Mycoplasma gallisepticum* is a bacterium that jumped from poultry to house finches (*Haemorhous mexicanus*) in the early 1990s in which it causes severe conjunctivitis (Ley et al., 1996; Hochachka et al., 2013) and can have severe impact on population size (Hochachka and Dhondt, 2000; Dhondt et al., 2006). As it spread across the continent *M. gallisepticum* rapidly evolved (Delaney et al., 2012; Tulman et al., 2012; Hochachka et al., 2013) and increased in virulence once endemic in a region (Hawley et al., 2013). Because haemosporidian parasites are frequently detected in house finches (Kimura et al., 2006; Davis et al., 2013), and given the increasing interest of possible effects of coinfection (Cressler et al., 2016) we tested to what extent chronic *Plasmodium* infections would impact house finches experimentally exposed to *M. gallisepticum*.

The objective of the experiment was to determine if (1) birds naturally infected with *Plasmodium* would become more rapidly infected with *M. gallisepticum* through horizontal transmission compared to individuals in which no *Plasmodium* was detected; (2) if birds infected with both *M. gallisepticum* and *Plasmodium* would develop more severe *M. gallisepticum*-induced clinical disease than birds infected only with *M. gallisepticum;* and (3) to what extent the response would vary with the virulence of the *M. gallisepticum* strain used.

2. Material and methods

Juvenile house finches were captured August–October 2015 in Ithaca, Tompkins County, New York (42°46′ N, 76° 45′ W) under permit (New York State Fish and Wildlife License 39, Albany, NY; and United States Geological Survey, Department of the Interior, Laurel, MD, permit 22669). Experiments were approved by Cornell University's IACUC protocol 2009-034.

Only birds negative for *M. gallisepticum* were used. Infection status was determined by visual inspection for eye lesions (Kollias et al., 2004), by Realtime Polymerase Chain Reaction (qPCR) designed to test for the presence of *M. gallisepticum* DNA from conjunctival swabs (Grodio et al., 2008), and by Rapid Plate Agglutination (RPA) to test for the presence of *M. gallisepticum*-specific antibodies in blood (Sydenstricker et al., 2006). We identified haemosporidian infection by lineage using DNA extracted from blood and using the nested polymerase chain (PCR) reactions described by Hellgren et al. (2004) and Waldenstrom et al. (2004) targeting the mitochondrial cytochrome *b* gene for *Plasmodium* and *Haemoproteus*. We repeated the PCR test three times for each

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sample. All PCR products positive for any haemosporidian infection were sequenced and the product compared to the Malavi database (Bensch et al., 2009).

Captive house finches were placed in six groups of 12 individuals housed in large semi-outdoor octagonal aviaries with a ground surface area of 6.87 m^2 and a volume of 17.87 m^3 (Dhondt et al., 2013). Each aviary contained water and a multiperch tube feeder that dispensed ad libitum a pelleted diet (Roudybush, Inc. Cameron Park, CA) (2/3) mixed with sunflower seeds (1/3). One artificial tree and multiple acrylic perches were distributed in identical fashion inside each aviary, as well as ceramic heat lamps. Five of the six groups consisted of six birds with and six without evidence of haemosporidian parasites and one group contained seven birds with and five without haemosporidian parasites.

To introduce M. gallisepticum in a group of birds we selected at random one group member with and one without Plasmodium and instilled 0.05 ml of M. gallisepticum inoculum in each conjunctival sac. We used three M. gallisepticum strains that differed in virulence (Hawley et al., 2013): CA2008 a weakly virulent strain, and NC2008 and CA2015 two more virulent ones. Because the number of colony forming units (CFU) varied between inocula, and as the CFU/ml was lowest for CA2008 at 6.20 × 10^6 CFU/ml, we diluted the other inocula with Frey's medium to equalize the CFU/ml in all three inocula. The precise identifiers for the inocula used are: CA2008-2008.028-2-3P; NC2008 2008.031-4-3P; CA2015−2015.002-3-2P. Each M. gallisepticum strain was introduced in two of the groups. The single group in which 5 birds were negative and 7 positive for Plasmodium was assigned to CA2008.

We examined the birds for eye lesions, and collected conjunctival swabs for qPCR on day 0, 4, 10, 17, 31, 38, 45, 52, and 59 following the introduction of M. gallisepticum in the group on 30 November 2015 (Day 0); we took a blood sample for antibody testing by RPA on Day PI (post introduction) 0, 17, 31, 45, and 59. Eye lesions were scored on a scale of 0 (no lesions visible) to 3 (severe lesions) for each eye (Sydenstricker et al., 2005). Eye scores used here are the sum of the score in each eye, with a combined maximum of 6. The experiment was terminated on day 59 PI.

Sampling for presence of M. gallisepticum DNA was done by swabbing the conjunctiva of both eyes of a bird using a separate sterile cotton tipped 3 inch wood handle swab for each eye (Fisher Scientific) that was then placed in 200 μl tryptose phosphate broth (TPB) and stored at −25 °C. DNA extraction from conjunctival swab samples was carried out using a Qiagen DNeasy blood and tissue kit (Qiagen, Valencia, California, USA), following the manufacturer's recommended protocol for the purification of total DNA from animal tissues. Conjunctival swabs were tested for the presence of M. gallisepticum DNA using qPCR as described by Grodio et al. (2008). For antibody testing blood samples taken from a bird's left brachial vein were collected into micro-capillary tubes. Serum was tested for M. gallisepticum antibodies by Rapid Plate Agglutination using commercially available M. gallisepticum antigen produced by Charles River Laboratories, Inc using the A5969 M. gallisepticum poultry strain.

2.1. Statistical methods

To determine the rate of horizontal transmission between the inoculated index birds and the naïve group members we used survival analysis in the Statistix10 software package, (Tallahassee, FL). For each naïve individual we determined the time to first evidence of infection as the Day PI that an individual developed any sign of infection: eye score (score >0), M. gallisepticum-DNA in the eye swab, or evidence of presence of antibodies if neither other sign of infection was observed. We used the Logrank test which allows for censored data, and report the χ^2 and P-values of the Logrank tests.

To test for effects of the M. gallisepticum-strain and of Plasmodium on M. gallisepticum-load and on eye scores we summed the 8 values of M. gallisepticum-load and eye lesions from day 4 to day 59 PI and used these values in a two-way Analysis of Variance (Statistix10 software package). M. gallisepticum-load values were log (value + 1)-transformed. We first calculated the model with an interaction term between Plasmodium and M. gallisepticum-strain. If the interaction term was not significant, we recalculated the ANOVA without it.

3. Results

3.1. Presence of haemosporidians in juvenile house finches

During our initial screening we detected three different Plasmodium haplotypes, but no Leucocytozoon nor Haemoproteus. BLASTN results of the sequences identified in the MALAVI database (Bensch et al., 2009) showed that out of 46 haplotypes PADOM11 (Plasmodium sp.) was found 35 times (76.1%), WW3 (Plasmodium sp.) was found 9 times (19.6%) and SEIAUR01 (Plasmodium cathemerium) was found twice (4.5%). We distributed the birds among the six groups to equalize the number of birds with each haplotype. Thus in 5 groups five birds were infected with PADOM11 and one bird with WW3; the sixth group, in which we introduced the M. gallisepticum CA2008 strain, had five birds with PADOM11, one with WW3 and one l bird with SEIAUR01.

3.2. Response of index birds to M. gallisepticum inoculation

The index birds are the initial source of the horizontal transmission in each group, although as other birds become infected with M. gallisepticum, these can also contribute to further transmission. To provide an idea about differences between the M. gallisepticum strains used in this experiment, and between birds with and without Plasmodium we summarize the mean summed M. gallisepticum-load across the 8 samples taken on days 4—59 PI for each index bird as shown in Table 1. In all aviaries there was a source of M. gallisepticum infection, although in one of the aviaries in which NC2008 was introduced only one index birds developed disease. Given the very small sample sizes we did not perform statistical tests to compare M. gallisepticum-load between isolates or birds with/without Plasmodium, although the data suggest that the three M. gallisepticum isolates differ in virulence as expected (CA2008 < NC2008 ≤ CA2015) and that birds with Plasmodium developed infections with a higher M. gallisepticum -load and tended to have more severe lesions.

3.3. Horizontal transmission and Plasmodium infection

3.3.1. Time to first infection

To determine the time to first infection through horizontal transmission of each naïve individual we determined what day post

Table 1
Mean and standard errors of M. gallisepticum-load summed to day 59 PI in index birds.

	CA2008	NC2008	CA2015
No Plasmodium (n = 2)	17.62 ± 1.17	19.26 ± 1.52	15.99 ± 2.05
Plasmodium (n = 2)	15.74 ± 9.80	21.65 ± 21.65[a]	33.08 ± 5.04

[a] One of the index birds, although it seroconverted, did not develop disease and hence would not have transmitted M. gallisepticum. The summed M. gallisepticum-load of the 2nd bird in the same aviary was 43.29.

Table 2
Mean and standard errors of eye scores (eye score) and *M. gallisepticum*-loads (MG load) summed to day 59 PI of house finches exposed to different *M. gallisepticum* isolates that had (Yes) or did not (No) have chronic *Plasmodium* infections before the start of the experiment. Groups of 12 birds were held in large aviaries into which *M. gallisepticum* was introduced. Sample sizes vary between groups because only birds that developed an infection are included in the analysis.

MG isolate	*Plasmodium* present	n	Eye score	MG load
CA2008	No	6	2.83 ± 1.25	1.53 ± 1.48
CA2008	Yes	4	1.00 ± 0.58	5.14 ± 5.10
NC2008	No	9	7.11 ± 2.34	7.11 ± 2.03
NC2008	Yes	7	11.43 ± 4.46	14.47 ± 4.92
CA2015	No	9	12.11 ± 3.54	18.73 ± 2.24
CA2015	Yes	9	24.89 ± 3.62	27.63 ± 2.57

Analysis of Variance Table for *M. gallisepticum*-load summed to day 59 PI					
Source	DF	SS	MS	F	P
Plasmodium	1	557.91	557.91	5.79	0.020
Isolate	2	2724.89	1362.44	14.15	0.0000
Error	40	3851.63	96.29		
Total	43				

Analysis of Variance Table for eye score summed to day 59 PI					
Source	DF	SS	MS	F	P
Plasmodium	1	453.43	453.427	5.20	0.028
Isolate	2	1706.66	853.330	9.79	0.0003
Error	40	3485.97	87.149		
Total	43				

introduction (PI) of *M. gallisepticum* in the aviary each individual developed any sign of infection: eye lesions (eye score >0), *M. gallisepticum*-DNA present in the eye swab, or evidence of presence of antibodies if neither other sign of infection was observed.

Survival analysis showed that the three *M. gallisepticum* strains differed significantly in transmission rates ($\chi^2 = 13.32$, df = 2, P = 0.0013). Transmission was significantly slower in the group with CA2008 than in both other groups (NC2008 versus CA2008: $\chi^2 = 8.39$, df = 1, P = 0.004; CA2015 versus CA2008: $\chi^2 = 8.20$, df = 1, P = 0.004), but there was no significant difference between CA2015

and NC2008 ($\chi^2 = 0.28$, df = 1, P = 0.60). The lower transmission in the groups exposed to CA2008 was primarily caused by the fact that by day 59 PI, when the experiment was terminated, half of the naïve birds still showed no signs of having become infected, while in the other groups more birds showed evidence of having been infected by *M. gallisepticum*.

While the time to infection did differ between groups exposed to different *M. gallisepticum* isolates, there was no effect of *Plasmodium*: birds with and without *Plasmodium* did not differ in time to infection in any of the groups (all P > 0.13).

3.3.2. Severity of disease if infected

Some birds did not develop any signs of infection. Because we wanted to address the question how birds responded if infected by horizontal transmission we used only those birds that either developed eye lesions or in which *M. gallisepticum*-load in the conjunctiva was non-zero. This reduced the sample sizes to 10/20 for CA2008, 16/20 for NC2008 and 18/20 for CA2015.

In order to answer questions 2 (effect of *Plasmodium*?) and 3 (effect of *M. gallisepticum* strain used?) we performed a two-way analysis of variance with interaction to determine if either *M. gallisepticum*-strain or the presence of *Plasmodium* impacted *M. gallisepticum*-load and/or eye lesions. Given that the interaction term was not significant for *M. gallisepticum*-load (P = 0.80) nor for eye lesions (P = 0.12) we only report the results without interaction (Table 2) and illustrate the change of *M. gallisepticum*-load and eye lesions for one *M. gallisepticum*-strain over time in Fig. 1. The results show that the response to infection through horizontal transmission in a group varied significantly between *M. gallisepticum*-strains to which the birds were exposed (P < 0.0001) but also that birds with a chronic *Plasmodium* infection developed more severe clinical disease (P < 0.03).

4. Discussion

House finches chronically infected with *Plasmodium* spp. and exposed to *M. gallisepticum* are equally likely to become infected with *M. gallisepticum* through horizontal transmission than group members without a chronic *Plasmodium* infection. A chronic *Plasmodium* infection thus does not make a bird more sensitive to
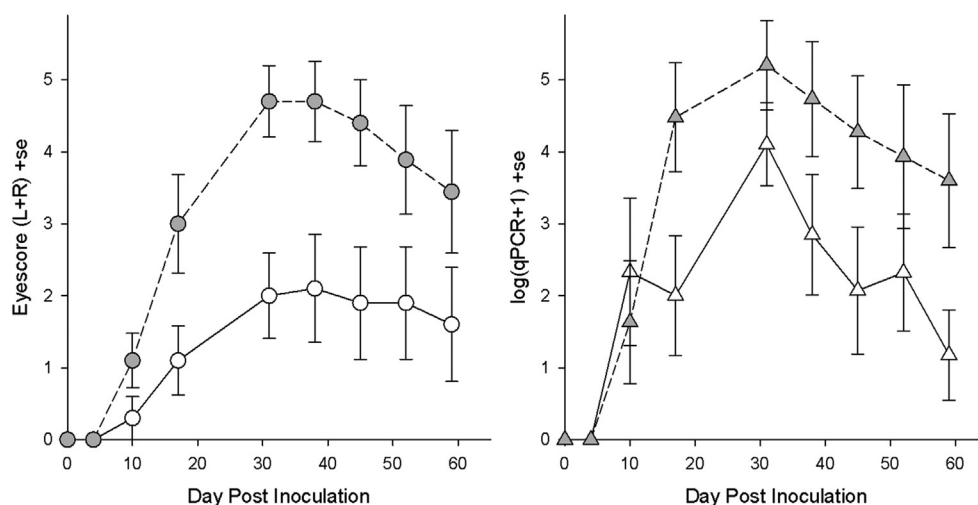

Fig. 1. Mean severity of eye lesions ± SE (circles), and *M. gallisepticum*-load (log (qPCR+1)) (triangles) of birds infected through horizontal transmission with the CA2015 strain of *M. gallisepticum*. Birds in which *Plasmodium* was detected by PCR (grey symbols) developed more severe disease than birds in which *Plasmodium* was not found (white symbol). As there is no significant interaction between *M. gallisepticum* −strain and *Plasmodium* infection (Table 2) the results for the other *M. gallisepticum* strains are qualitatively similar and therefore not shown.

infection. In this experiment one of the two birds that were the source of *M. gallisepticum* in each group had and one did not have a chronic *Plasmodium* infection. In order to determine the role of chronic *Plasmodium* infections on *M. gallisepticum* transmission one would need to compare groups in which both birds used to introduce *M. gallisepticum* in the aviary did not or did have chronic *Plasmodium* infections. The latter would deposit more *M. gallisepticum* on the feeder, which would have an effect on *M. gallisepticum* transmission rates (Adelman et al., 2013).

Although the *Plasmodium*-positive birds developed more severe clinical disease in response to the *M. gallisepticum* exposure, this could be attributed to other traits that correlate with both maintenance of a chronic *Plasmodium* infection and a stronger response to *M. gallisepticum* infection, and therefore this experiment, although suggestive, does not conclusively prove cause and effect. For example, social status or multilocus heterozygosity by influencing immunocompetence (Hawley et al., 2005, 2007) might alter the response to both infections. Experiments in which birds, naïve to both pathogens, are experimentally infected with *Plasmodium* and with *M. gallisepticum* are needed to confirm that the relationship found is causal.

Dhondt and Dobson (2017) speculated that in birds infected both with the widespread *Plasmodium* parasite and the emerging *M. gallisepticum* bacteria coinfection might facilitate transmission of both (Dhondt and Dobson, in press). Thus, if we can confirm that birds coinfected with *Plasmodium* carry higher *M. gallisepticum* loads for a longer period of time this could lead to higher transmission rates (Hawley et al., 2013; Williams et al., 2014) resulting in a higher proportion of the population being infected with *M. gallisepticum*. The observation that prevalence of mycoplasmal conjunctivitis increases from North to South among house finch population in eastern USA (Altizer et al., 2004) might in part be explained by higher *Plasmodium* parasitaemia in those regions (Davis et al., 2013). Reciprocally because house finches infected with *M. gallisepticum* transiently increase corticosterone levels (Love et al., 2016), they are more attractive to mosquitoes (Gervasi et al., 2016). The increased mosquito feeding rate could facilitate *Plasmodium* transmission. This remains to be tested experimentally.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2017.03.003.

References

Adelman, J.S., Carter, A.W., Hopkins, W.A., Hawley, D.M., 2013. Deposition of pathogenic Mycoplasma gallisepticum onto bird feeders: host pathology is more important than temperature-driven increases in food intake. Biol. Lett. 9, 20130594—20130594.

Altizer, S., Hochachka, W.M., Dhondt, A.A., 2004. Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. J. Anim. Ecol. 73, 309—322.

Asghar, M., Bensch, S., Tarka, M., Hansson, B., Hasselquist, D., 2015. Maternal and genetic factors determine early life telomere length. Proc. R. Soc. B-Biological Sci. 282.

Asghar, M., Palinauskas, V., Zaghdoudi-Allan, N., Valkiūnas, G., Mukhin, Platonova, E., Färnert, A., Bensch, S., Hasselquist, D., 2016. Parallel telomere shortening in multiple body tissues owing to malaria infection. Proc. R. Soc. B 283, 20161184.

Bensch, S., Hellgren, O., Perez-Tris, J., 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol. Ecol. Resour. 9, 1353—1358.

Cressler, C.E., McLeod, D.V., Rozins, C., van den Hoogen, J., Day, T., 2016. The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. Parasitology 143, 915—930.

Davis, A.K., Hood, W.R., Hill, G.E., 2013. Prevalence of blood parasites in eastern versus western house finches: are eastern birds resistant to infection? Ecohealth 10, 290—297.

Delaney, N.F., Balenger, S., Bonneaud, C., Marx, C.J., Hill, G.E., Ferguson-Noel, N., Tsai, P., Rodrigo, A., Edwards, S.V., 2012. Ultrafast evolution and loss of CRISPRs following a host shift in a novel wildlife pathogen, Mycoplasma gallisepticum. PLoS Genet. 8.

Dhondt, A.A., Badyaev, A.V., Dobson, A.P., Hawley, D.M., Driscoll, M.J.L., Hochachka, W.M., Ley, D.H., 2006. Dynamics of mycoplasmal conjunctivitis in the native and introduced range of the host. Ecohealth 3, 95—102.

Dhondt, A.A., Dhondt, K.V., Hochachka, W.M., Schat, K.A., 2013. Can american goldfinches function as reservoirs for mycoplasma gallisepticum? J. Wildl. Dis. 49, 49—54.

Dhondt, A.A., Dobson, A.P., 2017. Stress hormones bring birds, pathogens and mosquitoes together. Trends Parasitol. http://dx.doi.org/10.1016/j.pt.2017.01.001.

Gervasi, S.S., Burkett-Cadena, N., Burgan, S.C., Schrey, A.W., Hassan, H.K., Unnasch, T.R., Martin, L.B., 2016. Host stress hormones alter vector feeding preferences, success, and productivity. Proc. R. Soc. B 283, 20161278.

Grodio, J.L., Dhondt, K.V., O'Connell, P.H., Schat, K.A., 2008. Detection and quantification of Mycoplasma gallisepticum genome load in conjunctival samples of experimentally infected house finches (Carpodacus mexicanus) using real-time polymerase chain reaction. Avian Pathol. 37, 385—391.

Hawley, D.M., Jennelle, C.S., Sydenstricker, K.V., Dhondt, A.A., 2007. Pathogen resistance and immunocompetence covary with social status in house finches (Carpodacus mexicanus). Funct. Ecol. 21, 520—527.

Hawley, D.M., Osnas, E.E., Dobson, A.P., Hochachka, W.M., Ley, D.H., Dhondt, A.A., 2013. Parallel patterns of increased virulence in a recently emerged wildlife pathogen. PLoS Biol. 11.

Hawley, D.M., Sydenstricker, K.V., Kollias, G.V., Dhondt, A.A., 2005. Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. Biol. Lett. 1, 326—329.

Hellgren, O., Waldenstrom, J., Bensch, S., 2004. A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J. Parasitol. 90, 797—802.

Hochachka, W.M., Dhondt, A.A., 2000. Density-dependent decline of host abundance resulting from a new infectious disease. Proc. Natl. Acad. Sci. U. S. A. 97, 5303—5306.

Hochachka, W.M., Dhondt, A.A., Dobson, A., Hawley, D.M., Ley, D.H., Lovette, I.J., 2013. Multiple host transfers, but only one successful lineage in a continent-spanning emergent pathogen. Proc. R. Soc. B-Biological Sci. 280.

Kimura, M., Dhondt, A.A., Lovette, I.J., 2006. Phylogeographic structuring of Plasmodium lineages across the north american range of the house finch (Carpodacus mexicanus). J. Parasitol. 92, 1043—1049.

Knowles, S.C.L., Palinauskas, V., Sheldon, B.C., 2010. Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. J. Evol. Biol. 23, 557—569.

Kollias, G.V., Sydenstricker, K.V., Kollias, H.W., Ley, D.H., Hosseini, P.R., Connolly, V., Dhondt, A.A., 2004. Experimental infection of house finches with Mycoplasma gallisepticum. J. Wildl. Dis. 40, 79—86.

Ley, D.H., Berkhoff, J.E., McLaren, J.M., 1996. Mycoplasma gallisepticum isolated from house finches (Carpodacus mexicanus) with conjunctivitis. Avian Dis. 40, 480—483.

Love, C.L., Foltz, S.L., Adelman, J.S., Moore, I.M., Hawley, D.M., 2016. Changes in corticosterone concentrations and behavior during *Mycoplasma gallisepticum* infection in house finches (Haemorhous mexicanus). General Comp. Endocrinol. 235, 70—77.

Marzal, A., Asghar, M., Rodriguez, L., Reviriego, M., Hermosell, I.G., Balbontin, J., Garcia-Longoria, L., de Lope, F., Bensch, S., 2013. Co-infections by malaria parasites decrease feather growth but not feather quality in house martin. J. Avian Biol. 44, 437—444.

Merino, S., Moreno, J., Sanz, J.J., Arriero, E., 2000. Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (Parus caeruleus). Proc. R. Soc. B-Biological Sci. 267, 2507—2510.

Sydenstricker, K.V., Dhondt, A.A., Ley, D.H., Kollias, G.V., 2005. Re-exposure of captive house finches that recovered from *Mycoplasma gallisepticum* infection. J. Wildl. Dis. 41, 326—333.

Sydenstricker, K.V., Dhondt, A.A., Hawley, D.M., Jennelle, C.S., Kollias, H.W., Kollias, G.V., 2006. Characterization of experimental Mycoplasma gallisepticum infection in captive house finch flocks. Avian Dis. 50, 39—44.

Tulman, E.R., Liao, X., Szczepanek, S.M., Ley, D.H., Kutish, G.F., Geary, S.J., 2012. Extensive variation in surface lipoprotein gene content and genomic changes

associated with virulence during evolution of a novel North American house finch epizootic strain of *Mycoplasma gallisepticum*. Microbiology-Sgm 158, 2073—2088.

Waldenstrom, J., Bensch, S., Hasselquist, D., Ostman, O., 2004. A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. J. Parasitol. 90, 191—194.

Williams, P.D., Dobson, A.P., Dhondt, K.V., Hawley, D.M., Dhondt, A.A., 2014. Evidence of trade-offs shaping virulence evolution in an emerging wildlife pathogen. J. Evol. Biol. 27, 1271—1278.