Expression of immune genes RIG-I and Mx in mallard ducks infected with low pathogenic avian influenza (LPAI): A dataset

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This article provides data on primer sequences used to amplify the innate immune genes RIG-I and Mx and a set of normalizing reference genes in mallards (Anas platyrhynchos), and shows which reference genes are stable, per tissue, for our experimental settings. Data on the expresional changes of these two genes over a time-course of infection with low pathogenic avian influenza virus (LPAI) are provided. Individual-level data are also presented, including LPAI infection load, and per tissue gene expression of RIG-I and Mx. Gene expression in two outlier individuals is explored in more depth.

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Value of the data

- Avian influenza virus (AIV) infection of mallards was achieved via a semi-natural, contact infection route to mimic natural transmission of the virus.
- Infection with low pathogenic AIV provides a contrast to most previous studies that used highly pathogenic AIV to study immune gene expression in mallards.
- A set of reference genes that had been experimentally validated as stable under the given experimental treatment were used to stabilize RT-qPCR.
- A table summarizing the methodology and findings of previous studies of Mx and/or RIG-I expression in AIV infected ducks is provided.

1. Data

The dataset provided here provides additional information for Helin et al. [1]. In that paper, we show that the innate immune genes retinoic acid-inducible gene-I (RIG-I) and myxovirus resistance gene (Mx) are rapidly yet transiently upregulated after infection with low pathogenic avian influenza virus (LPAI) subtype H1N1. Helin et al. aims to provide a series of methodological improvements over previous analyses of immune gene expression in ducks infected with avian influenza virus (AIV).

Table 1 shows that most previous studies have used highly pathogenic avian influenza virus (HPAI), which is rarely detected in wild mallards [2,3]. Additionally, infection in previous studies was achieved via artificial inoculation comprising potentially unnatural viral doses and infection routes. These previous studies have almost exclusively been conducted on domestic Pekin ducks, rather than the main wildlife reservoir for avian influenza, mallard ducks (Anas platyrhynchos). Lastly, most previous studies have used a single, non-validated reference gene (often GAPDH) for normalizing gene expression data. This approach leads to potentially misleading interpretation of data [4].
Table 1

| Innate Gene | Strain | Viral dose | Innoculation Method | Tissuesanalysed | RG | Timepoints | N. individsg | Breed | Result\(^f\) | Refs. |
|-------------|--------|------------|---------------------|----------------|----|------------|-------------|-------|-------------|-------|
| RIG-I       | HPAI   | H5N1\(^a\) | 10^5 of EID\(_{50}\) Dripped into nares, eyes & trachea | Lung, intestine | GAPDH | 1, 3 dpi | 3 | Pekin | Lung: ~200-fold at 1dpi, ~20-fold at 3 dpi Intestine: ~5-fold at 1dpi, ~2.5-fold at 3 dpi | [6] |
| RIG-I       | LPAI   | H5N2      | 10^6 of EID\(_{50}\) Dripped into nares, eyes & trachea | Lung, intestine | GAPDH | 1, 3 dpi | 2-3 | Pekin | No significant changes | [6] |
| RIG-I       | HPAI   | H5N1      | 10^5 of EID\(_{50}\) Intranasal | Spleen | β-actin\(^b\) | 2 dpi | 4 | Pekin | 13-fold | [7] |
| RIG-I       | LPAI   | H7N1      | 2 x 10^5 of EID\(_{50}\) Dripped intranasally & intratracheal | Lung, bursa, ileum | 18S\(^c\) | 0.8, 2, 4, 7, 14 dpi | 6 | Pekin | Spleen: ~10-fold at 1 & 2 dpi, 2-4-fold at 3 & 4 dpi Lung: ~2.5-fold at 0.8 dpi at 1, 2, 3, 4, 5, 7 dpi | [8] |
| RIG-I       | HPAI   | H7N1      | 2 x 10^5 of EID\(_{50}\) Dripped intranasally & intratracheally | Lung, brain, spleen | 18S | 0.3, 1, 2, 3, 4, 5, 7 dpi | 6 | Pekin | Spleen: ~65-fold in 5wk old ducks, ~4-fold in 2wk old ducks Lung: ~10-fold at 1 & 2 dpi, ~6-fold at 3 & 4 dpi | [9] |
| RIG-I       | HPAI   | H5N1\(^a\) | 10^5 of EID\(_{50}\) Intranasal | Spleen, lung | β-actin | 2 dpi | 4 | Pekin | Lung: ~65-fold in 5wk old ducks, ~2-fold in 2wk old ducks | [10] |
| Mx          | LPAI   | recombinant | 0.1 MOI Cells & virus mixed together | Embryo fibroblast cells | GAPDH | 2, 4, 8, 12, 24 hpi | NA | Pekin | ~500-1000-fold at 8-24 hpi | [11] |
| Mx          | HPAI   | H5N1      | 1.0 MOI Cells & virus mixed together | Peripheral blood mononuclear cells | GAPDH | 4, 8, 12, 24, 36, 48 hpi | NA | Mallard | 25-40-fold at 8-24 hpi | [12] |
| Mx          | LPAI   | H1N1      | 0.1 MOI Cells & virus mixed together | Primary lung cells | GAPDH | 12, 24, 48 hpi | NA | Pekin | No significant changes | [13] |
| Mx          | LPAI   | H5N9      | 0.1 MOI Cells & virus mixed together | Primary lung cells | GAPDH | 12, 24, 48 hpi | NA | Pekin | ~5-fold at 12 hpi, 12-fold at 24 hpi, ~8-fold at 48 hpi | [13] |
| Mx          | LPAI   | H7N1      | 10^7 PFU Intrachoanal cleft & oral | Illeum | GAPDH | 1, 6 dpi | 6-7 | Pekin | Upregulation at 1 & 6 dpi\(^i\) | [14] |
| Mx          | LPAI   | H7N1      | 10^7 PFU Intrachoanal cleft & oral | Illeum | GAPDH | 1, 6 dpi | 3| Pekin | Upregulation at 1 & 6 dpi\(^i\) | [15] |

\(^a\) Three strains, derived from chicken, egret and duck.
\(^b\) Authors state β-actin was stable between uninfected and infected, but no details given and no other RGs investigated.
\(^c\) Authors state that 18S had the most stable expression over time and between tissues in ducks, but data is not shown and no indication of which RGs were compared.
\(^d\) Five control individuals.
\(^e\) Many results were inferred from graphs because exact results were not listed. In such cases, ~ is used to indicate fold changes are approximate.
\(^f\) Results not expressed as fold-change. Significant upregulation with one of the two tested viruses only.
2. Experimental design, materials and methods

To address these methodological issues, in Helin et al. [1] we use a semi-natural infection regime to infect mallards with low pathogenic H1N1 AIV. We then use a set of reference genes (Tables 2 and 3), that we have previously demonstrated to be stable under these experimental settings [5], to normalize RT-qPCR data. A full description of the experimental design, materials and methods is provided in Helin et al. [1].

Datasets describing the fold-change in expression between experimental time-points, and per individual, for each tissue type and gene are provided as Supplementary tables S1–4 and Figs. S1–S4 to this article. Fig. S5 provides a more in-depth analysis of two individuals with extremely high expression, showing that this over-expression was restricted to a specific tissue and a single gene at single time-point.

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Transparency document. Supporting information

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.04.061.

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