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Examination of the Adaptive Immune Response to *Syngamus trachea* in the House Sparrow

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ABSTRACT

Due to global warming and other anthropogenic factors, host-parasite relationships are changing. To understand the causes and be prepared for the consequences of these changes, it is essential to study the mechanisms of host-parasite relationships further. In this study, the adaptive immune response of the house sparrow (*Passer domesticus*) against gapeworm (*Syngamus trachea*) was investigated. The goal was to discover how both the total and the *S. trachea* antigen-specific plasma levels of three immunoglobulins (IgY, IgM and IgA), are related to *S. trachea* infection status, age, geographical location, and faecal egg count (FEC). The methods used were direct ELISA and indirect ELISA. The results showed that the concentration of *S. trachea*-specific IgY was higher in infected than in non-infected birds. Additionally, the total levels of IgY and IgM were higher in a farm-dwelling population than in a population not on a farm, suggesting that pathogen levels affect the total plasma concentration of these immunoglobulins. Lastly, juveniles had a higher total IgM plasma concentration, but a lower *S. trachea*-specific IgY concentration. These findings show that the adaptive immune system, particularly IgY, is involved in the immune response of house sparrows to *S. trachea*, and suggest that adults have higher immunity to *S. trachea* than juveniles because of earlier exposure. Future studies should look into the genetic diversity at immunoglobulin loci, to determine whether they are targets of selection and have evolutionary importance. Knowing more about host-parasite relationships on a molecular level is an important part of developing new methods of ecosystem monitoring and control in a changing world.

SAMMENDRAG

Global oppvarming og andre antropologiske faktorer gjør at forhold mellom parasitter og vertsorganismer endrer seg. For å forstå årsakene til og for å være forberedt på konsekvensene av dette er det essensielt å studere mekanismene som ligger bak vert-parasitt-forhold. I dette studiet ble den adaptive immunresponsen hos gråspurv (*Passer domesticus*) mot parasitten *Syngamus trachea* undersøkt. Formålet var å finne ut hvordan både det totale immunoglobulin-nivået og nivået av *S. trachea*-spesifikke immunoglobuliner i plasma relaterte seg til infeksjonsstatus, alder, geografisk plassering og antall egg i avføring (FEC). Immunoglobulinene som skulle undersøkes var IgY, IgM og IgA. Immunoglobulinkonsentrasjonene ble målt ved hjelp av direkte ELISA og indirekte ELISA. Resultatene viste at konsentrasjonen av *S. trachea*-spesifikk IgY var høyere hos fugler smittet med parasitten enn hos friske fugler. De totale plasmakonsentrasjonene av IgY og IgM var høyere hos en populasjon som holdt til på gårder enn hos en populasjon som ikke gjorde det, noe som antyder at mengden patogener i habitatet påvirket den totale konsentrasjonen av disse immunoglobulinene. I tillegg hadde juvenile fugler en høyere total konsentrasjon av IgM, men en lavere konsentrasjon av *S. trachea*-spesifikk IgY. Disse funnene viser at det adaptive immunforsvaret, særlig IgY, er involvert i immunresponsen mot *S. trachea* hos gråspurv, og de indikerer også at voksne fugler har høyere immunitet mot *S. trachea* på grunn av tidligere utsettelse for parasitten. Fremtidige studier bør undersøke genetisk diversitet på loci knyttet til immunoglobuliner for å finne ut om de blir selektert for og dermed er viktige for evolusjonen. Å finne ut mer om vert-parasitt-forhold på et molekylært nivå er en viktig del av utviklingen av nye metoder for å overvåke og kontrollere økosystemer i en verden i stadig endring.

1 TABLE OF CONTENTS

2 INTRODUCTION

2.1 GLOBAL CHANGE AND PARASITES

In today's world, many anthropogenic factors contribute to the changing of ecosystems, both indirect - such as global human population growth - and direct - such as increased land use for agriculture, deforestation, and the release of CO_2 from the burning of fossil fuels (Nelson et al., 2006). Both the release of CO_2 and deforestation contribute to climate change, which is an increasingly important driver of ecosystem changes (Allen et al., 2018; Staudt et al., 2013).

One of the biological processes affected by these global changes is the relationship between parasites and their hosts. For example, Hudson et al. (2006) found that parasite development rate increases with temperature, and speculate that extreme climatic events will lead to an increase in the frequency and intensity of disease outbreaks. In facing these changes, it is important to gain a better understanding of host-parasite relationships, such as the molecular mechanisms of host defence and how parasites respond to and regulate the host's immune system (Maizels & McSorley, 2016). Gaining more insight into these mechanisms is the first step in better understanding the ecological and evolutionary consequences of global changes for both host and parasite populations. Climate change is likely to affect different host-parasite relationships differently (Merino & Møller, 2010), so it is difficult to make general statements about how they will change. Some parasites will become more prevalent and others less, as there are many direct and indirect factors involved in each host-parasite relationship. This is why it is important to study different host-parasite interactions in natural populations to gain a better understanding of the effects ecosystem changes have on such interactions in nature (Staudt et al., 2013). Additionally, resistance to the anthelmintic drugs used in agriculture is becoming more prevalent in parasite populations, and other, sustainable ways of controlling infection rates are needed (Jackson & Miller, 2006). Hence, research into the molecular processes underlying host-parasite relationships is important for the development of new sustainable methods of parasite control (Jackson & Miller, 2006).

Parasitic helminths such as nematodes cause significant morbidity and mortality among animals worldwide (Jex et al., 2013). Their life cycle generally consists of a larval stage, in which they are ingested by the host and penetrate the mucosa of either the stomach or intestine before going through several moults. After approximately three weeks, the larvae reach adulthood and

eventually lay eggs that are excreted in the host faeces (Williams, 2011). In sheep, young lambs are more susceptible to nematode infection than adults, and infection significantly reduces their growth rates. Different nematode species cause different symptoms, such as anaemia and diarrhoea, but a general sign of infection is a reduction in appetite, which causes the reduction in growth rate (Williams, 2011).

Studies done on parasite-host interactions that involve parasitic nematodes often use faecal egg counts (FEC) as a measurement of parasite load. This is a time and resource-consuming method, so there have been investigations into using the measurement of plasma immunoglobulin concentration as an alternative (Aboshady et al., 2020). This could make it easier to study host-parasite interactions because of sampling considerations. Furthermore, if plasma immunoglobulin concentrations reflect parasite infection status or parasite load, this would potentially also allow examination of causes and consequences of parasite infection at an individual level across time and space.

As this study concerns the adaptive immune response of house sparrows, the following sections will focus primarily on avian responses to parasites, though many of them are similar to those of other vertebrates. Birds are exposed to many types of parasites, both internal and external, and have developed several anti-parasite defences. The first line of defence is behavioural, such as body maintenance, avoidance of infected prey, and tolerance (Bush & Clayton, 2018). The next defence against parasites is the immune response. The immune response against several helminth parasites such as nematodes is called the type 2 response, or T_H2 (T helper 2) response, and is an immunological pathway involving an increase of interleukin-4 and other T_H2-type cytokines (Anthony et al., 2007). This response has mostly been researched in mammals, but birds have it as well (Degen et al., 2005). The general T_H2 response involves both the innate and the adaptive immune systems (Anthony et al., 2007).

The innate immune system is an immune system not specific to particular pathogens but rather relies on proteins and phagocytic cells that recognise pathogens and are rapidly activated to destroy them (Alberts et al., 2002b). It is more primitive than the adaptive immune system and is the main immune system in plants, fungi, insects, and primitive multicellular organisms (Janeway et al., 2001). In vertebrates, the innate immune system is the first line of defence against pathogens, and it can ward off an infection until the adaptive immune system is activated (Alberts et al., 2002b). Lundregan et al. (2020) have already found genes linked to the innate

immune response to play a role in resistance to *S. trachea* in house sparrows, and their results indicated that the adaptive immune system is involved as well. That is why this study aims to further investigate the role of the adaptive immune system in *S. trachea* resistance.

2.2 THE ADAPTIVE IMMUNE SYSTEM

Immunoglobulins (Ig), also called antibodies (Ab), are proteins that bind to pathogens to identify or neutralise them. Immunoglobulins are a major constituent of the adaptive immune system, along with B and T cells. They are secreted from B cells that have turned into plasma cells from being activated by an antigen (Alberts et al., 2002a). Immunoglobulins exist both in a soluble form in extracellular fluids and attached to B cells. An antigen (Ag) is a molecule or other structure that can bind to an immunoglobulin or a B- or T-cell receptor. They are usually proteins on the surface of pathogens. While B cells capable of making immunoglobulins are produced in the bone marrow in mammals, in birds they are produced in the bursa of Fabricius (Davidson, 2014).

Immunoglobulins are large Y-shaped proteins. Their attachment to an antigen is formed between a site called the paratope on the immunoglobulin and the epitope site on the antigen. Paratopes can be found on the two tips of the Y-shaped immunoglobulin and are specific to one particular antigen. These paratopes can vary greatly, a fact that allows the immune system to recognise millions of different antigens. The rest of the immunoglobulin, however, only comes in a few different forms. These define the class or isotype of the immunoglobulin. The different isotypes found in mammals are IgA, IgD, IgE IgG and IgM. IgY is an isotype found in birds, amphibians and reptiles, while IgW is found in sharks and skates (Berstein et al., 1996; Lundqvist et al., 2006). The main antibody isotypes in birds are IgA, IgM and IgY (Härtle et al., 2014). Avian IgA and IgM have similar roles to their mammalian counterparts. That is, IgM is the main antibody to be attached to the surface of B cells, and IgA is the predominant isotype in secretions. IgY is similar to mammalian IgG in that it is the predominant isotype in secondary antibody responses, meaning the immune response occurring on the second and subsequent exposures to an antigen (Härtle et al., 2014).

Immunoglobulins consist of polypeptide chains called light chains and heavy chains. These chains consist of domains, either variable or constant (Figure 2.1). In a light chain, there is one variable domain (V_L) and one constant domain (C_L). A heavy chain consists of one variable

domain (V_H) and three to four constant domains (C_H1, C_H2, …). Structurally, immunoglobulins are divided into several parts, the two antigen-binding regions (Fab) and the crystallisable fragment (Fc) which determines the isotype of the Ig (Figure 2.1) (Barclay, 2003). Loci that code for heavy and light chains contain clusters of different genes called V, J, C and D genes, organised differently at different loci.
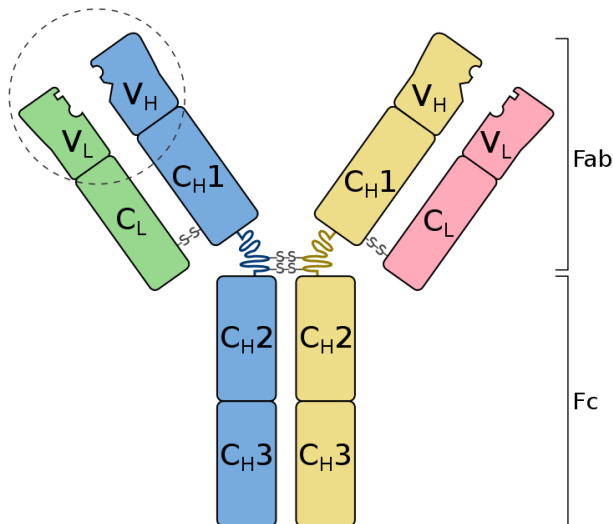

Figure 2.1: Schematic depiction of an immunoglobulin, consisting of two heavy chains (blue and yellow) and two light chains (green and pink). The domains that make up the chains are depicted as rectangles and one of the antigen-binding regions is circled. V_L = light chain variable domain, V_H = heavy chain variable domain, C_L = light chain constant domain, C_H1, C_H2, … = Heavy chain constant domain 1, 2, …, Fab = antigen-binging regions, Fc = crystallisable fragment. *Image credits: see section 7.*

Through recombination, Igs with an immense paratope diversity in their variable regions are produced in B-cells and bind to their surface as a part of B-cell receptors (BCRs) (Mikocziova et al., 2021). The resulting diverse repertoire of immunoglobulins provides Igs that can bind to virtually any pathogen antigen in the body. Before the introduction of a novel pathogen, these Igs are only present at low levels (Mikocziova et al., 2021), but when one binds to an antigen, its proliferation is triggered by the following process: When an antigen binds to a BCR, it is endocytosed by the B-cell and processed into peptide fragments. Still within the B-cell, the peptide fragments are transported to late endosomal compartments containing MHC class II molecules, called the MIIC (Duffy et al., 2017; Stern et al., 2006). In the MIIC, the peptide fragments bind to MHC class II molecules in a process dependent on their affinity to the MHC class II molecule, as well as the kinetic stability of the resulting MHC class II peptide complex (Duffy et al., 2017). The complexes containing the "best fit" peptides are then transported to the B-cell surface, where they are recognised by T-cells, triggering a clonal expansion of the B-cells and T-cells that can bind to the relevant peptide antigen (Adler et al., 2017; Duffy et al.,

2017). Because each MHC class II molecule can only bind a limited number of antigens, it is beneficial to be polymorphic at the MHC locus as this gives resistance to a higher number of pathogens. Borg et al. (2011) found the levels of MHC variation in an inbred sparrow population to be high, and comparable to an outbred population in the same area, even though the inbred population had a low level of variation at neutral loci. This suggested that there was a high selection pressure maintaining a high level of variation at the MHC locus in these populations. Ultimately, the diversity of both immunoglobulin paratopes and MHC class II molecules is closely linked to the success of an adaptive immune response to a pathogen.

2.3 ENZYME-LINKED IMMUNOSORBENT ASSAY

The enzyme-linked immunosorbent assay (ELISA) is an analytical biochemistry assay used to detect and measure the concentration of a specific protein in a sample. A common application of ELISA is determining the concentration of a particular antibody in a plasma sample.

There are several types of ELISAs, the most basic method being direct ELISA. In a direct ELISA, a solution containing the antigen to be detected or measured is added to the wells of a microtiter plate (Figure 2.2). The antigen solution can be crude blood serum or plasma, containing the antigen to be detected, e.g., an immunoglobulin (the immunoglobulin is the antigen in this case, as it is detected by being bound by another immunoglobulin). Then a blocking solution is added to each well to prevent subsequent binding of proteins to the well surfaces. After this, a primary enzyme-conjugated detection antibody is added to the wells. Then a solution containing the enzyme's substrate is added, and an output is produced, such as a colour change. The strength of this output is proportional to the concentration of the antigen in each well, and the degree of binding between the primary antibody and the antigen. In a quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve - made from known concentrations of the antigen - to determine its concentration.
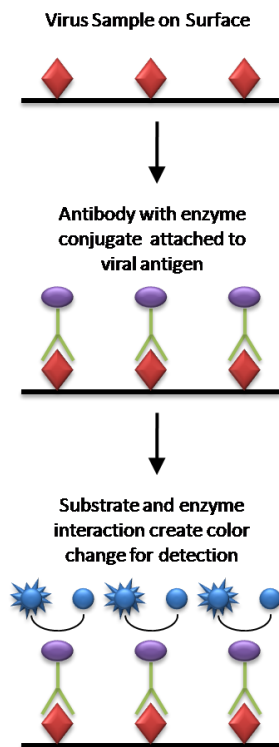
Virus Sample on Surface

Antibody with enzyme conjugate attached to viral antigen

Substrate and enzyme interaction create color change for detection

Figure 2.2: Direct ELISA with a virus sample as the antigen. In the present study, the antigens attached to the well surface will be sparrow immunoglobulins. The enzyme-conjugated detection antibodies will be specific to the sparrow Igs. *Image credits: see section 7.*

An indirect ELISA includes a secondary antibody that is specific to the primary antibody. In an indirect ELISA, the concentration of primary antibody is what is measured, and the secondary antibody is the enzyme-conjugated detection antibody. The indirect ELISA performed in the present study was for the detection of parasite antigen-specific immunoglobulins that bind to the parasite antigens attached to the well surface. As the concentration of antigen-specific Igs is lower than the total Ig concentration in plasma, they could be difficult to detect if the plasma samples or parasite antigen extract is too diluted. Thus, the volumes of plasma and parasite extract available are limiting factors.

For both direct and indirect ELISAs, the detection antibody (the primary antibody in direct, and secondary antibody in indirect ELISA) must have a high enough affinity to the antigen or primary antibody of interest. However, there are limitations to which antibodies are commercially available, which means that it is not always possible to use a species-specific detection antibody. In this study, anti-chicken detection antibodies were used in both ELISAs, as Fassbinder-Orth et al. (2016) found it to have nearly as high a detection rate in Passeridae birds as anti-passerine antibodies in direct ELISA. Fassbinder-Orth et al. (2013) successfully used anti-bird antibodies in an indirect ELISA, and as anti-chicken antibodies gave a much

higher detection rate than anti-bird antibodies in a direct ELISA (Fassbinder-Orth et al., 2016), they were chosen for the present study.

2.4 *SYNGAMUS TRACHEA* AND THE STUDY POPULATION

The gapeworm (*Syngamus trachea*) is a parasitic nematode that infects the trachea of several bird species. The infection results in blockage of the bird's airway, leading to the characteristic symptom of "gaping". The life cycle of *S. trachea* consists of egg, larva, and adult stages. The adult parasites live in the trachea of birds and produce eggs by sexual reproduction. These eggs are coughed up by the bird, then swallowed so they end up in the faeces. Intermediate invertebrate hosts, such as earthworms, eat the eggs, and then the *S. trachea* larvae hatch and form cysts in the muscle tissue of the intermediate host. When a bird (the definitive host) eats an infected intermediate host, the larvae emerge from the cysts, pass through the intestinal mucosa, and travel to the lungs through the bloodstream. In the trachea or bronchi they grow into adults, and the cycle starts again (Morgan & Clapham, 1934; Nevarez et al., 2002). Effects of the infection include rapid weight loss caused by respiratory distress, as well as pneumonia caused by larval migration from the lungs to the trachea (Andreopoulou et al., 2011). Accordingly, body condition has been found to be negatively associated with *S. trachea* infection in ring-necked pheasants (*Phasianus colchicus*) (Gethings et al., 2016). The host-mediated immune response to worms in the trachea also leads to a large increase in mucus that can potentially cause asphyxiation, especially in juveniles or birds with a heavy parasite load (Clapham, 1939; Gethings, 2018).

The house sparrow (*Passer domesticus*) is a small passerine bird that has been widely used as a model organism in fields such as ecology, evolution, and genetics (Hanson et al., 2020), for example to study behavioural ecology (Rohwer, 1975; Sanchez-Tojar et al., 2018; Veiga, 1996) and epigenetics (Hanson et al., 2020; Kilvitis et al., 2017; Schrey et al., 2012; Sheldon et al, 2018). A natural house sparrow metapopulation on the Helgeland coast in Northern Norway has been monitored since 1993, and individual data on for example morphology, survival and reproduction is collected every year. The sparrows in the metapopulation live closely associated with human settlements, and their diet includes cattle food and crop seeds as well as insects in the summer (Anderson, 2006; Holand et al., 2013). The samples used in this study were taken from a sub-set of the birds that were captured during one season (collected from July to August)

in the study metapopulation. This was because plasma samples had not been collected in previous years and should also be relatively fresh when analysed.

Several studies have examined the relationship between house sparrows and *S. trachea* in the Helgeland metapopulation. They have found that annual survival is lower in house sparrows with severe *S. trachea* infections that are exhibiting the gaping symptom (Holand et al., 2014). Infection also has negative effects on the reproductive success of female house sparrows, both in the short and long term (Holand et al., 2015). Additionally, the host-parasite relationship between the house sparrow and *S. trachea* is affected by temperature: Holand et al. (2019) found a positive relationship between parasite prevalence and temperature in the house sparrow metapopulation in Helgeland. Furthermore, Lundregan et al. (2020) found a genetic component to *S. trachea* susceptibility in the house sparrow. These studies show that *S. trachea* infection is widespread in house sparrows and that the relationship between the house sparrow and *S. trachea* is affected by both genetics and the sparrows' external environment, and that infection by this parasite affects individual fitness. This makes the house sparrow an ideal species in which to investigate the relationship between immunoglobulin levels and parasite load.

2.5 PREVIOUS RESEARCH AND REASONING BEHIND THIS STUDY

Windisch and Hess (2010) described the varying levels of antibodies in the chicken intestine during an experimental infection with the protozoan parasite *Histomonas meleagridis*. They found that IgY (called IgG) had the highest overall concentration, with a steady increase throughout the infection. Meanwhile, IgA and IgM concentrations remained lower and peaked on day 28 of the infection. Williams and Behnke (1983) found IgG levels to rise dramatically in response to immunizing infection with the parasite *Nematospiroides dubius* in mice, and a review by Aboshady et al. (2020) found that IgG levels generally increase following gastrointestinal nematode infection in sheep. In lambs, nematode-specific IgM increased after infection with gastrointestinal nematodes (Aboshady et al., 2020), and in the spotted hyena, both total IgG and IgA were higher in juveniles than in adults (Ferreira et al., 2021). Based on this, we might expect IgY levels to be higher in infected than non-infected house sparrows, both in total and of IgY specific to the parasite, and we may expect that IgY levels are higher in juveniles than in adults. Concerning the relationship between immunoglobulin concentration and parasite load, previous studies have found negative relationships between FEC and immunoglobulin concentrations, such as increased parasite-specific IgA and IgG predicting

lower FEC (Sparks et al., 2018) and an increase in total IgE being associated with lower FEC (Aboshady et al., 2020) in sheep. But positive relationships have also been found, such as the total IgA and IgG increasing with FEC in the spotted hyena (Ferreira et al., 2021), and parasite-specific IgG being positively related to FEC in horses (Kjaer et al., 2007). As both positive and negative relationships have been found, it is uncertain which relationship will be seen in the present study. However, FEC is a noisy measurement due to differences in parasite load at different stages of infection, and this may affect the results.

A study by Fassbinder-Orth et al. (2013) focused on differences in immune responses between a native (cliff swallow) and invasive (house sparrow) species. They successfully used an indirect ELISA to measure their parasite antigen-specific immunoglobulin levels, demonstrating that this method can be used in birds, and in house sparrows in particular.

It has previously been demonstrated that genes associated with the adaptive immune response are linked to *S. trachea* resistance in the house sparrow (Lundregan et al., 2020). The present study looks further into this by aiming to determine the relationship between the adaptive immune response and *S. trachea* infection. This was done by measuring the total concentrations of IgY, IgM and IgA in plasma from both infected and non-infected sparrows, and determining the relationship between immunoglobulin concentrations and both infection status and faecal egg count (FEC). In this study, I pose the following research questions: i) Do Ig levels vary between islands or by age of birds, ii) what are the relationships between *S. trachea* infection and parasite load and plasma immunoglobulin concentrations in the house sparrow, and iii) which immunoglobulin isotype or isotypes bind to *S. trachea* antigens in the house sparrow?

3 MATERIALS AND METHODS

3.1 COLLECTION OF PLASMA AND FAECAL SAMPLES

Adult and fledged juvenile house sparrows were captured using mist nets on the islands of Hestmannøy and Træna on the coast of Helgeland (66°50'N, 12°50'E) in July and August of 2021. Caught sparrows were placed in paper bags with small holes to allow air to enter. After 10-15 minutes, the birds were removed from the bags, and a faecal sample was collected from each bag. The faecal sample from each bird was transferred to a 1.5 mL Eppendorf tube containing distilled H_2O, and the samples were stored at 4 °C. The tarsus of each leg and the body mass were then measured. The measurements by each fieldworker were standardised by adjustment based on their difference from the measurements of T. H. Ringsby by using regression techniques (see Jensen et al., 2004). A blood sample of about 150 μL was taken from the brachial vein of each bird, then centrifugation was used to separate plasma from the other blood components. To extract blood from a bird, the brachial vein of one wing was punctured with a hypodermic needle and blood was gathered with two micro haematocrit tubes (75 μL) (Figure 3.1). If enough blood was not obtained from the puncture, blood was also collected from the other wing. The maximum amount of blood extracted from any bird was 200 μL, which is equivalent to approximately 0.7 % of the body weight. Subsequently, one end of each micro haematocrit tube was blocked with sealing clay, then the tubes were centrifuged at 13000 RPM for 1 minute in a capillary tube centrifuge. Each micro haematocrit tube was then cut with pliers, and the separated plasma was transferred to a 1.5 mL Eppendorf tube. Plasma samples were stored at -20 °C for 1-2 months (while still at Træna or Hestmannøy and during transport), then at -80 °C until analysis (2-6 months).

Figure 3.1: Blood sampling of a house sparrow. After puncture of the brachial vein, a drop of blood was allowed to form before it was collected with two micro haematocrit tubes.

3.2 FAECAL EGG COUNT

The number of *S. trachea* eggs in each faecal sample was determined by sucrose floatation (see also Holand et al., 2013): First, the samples were centrifuged at 3000 rpm for 1 minute. Then the supernatant was removed, and 1-1.5 mL sucrose-saturated water was added. Next, the samples were centrifuged at 1500 rpm for 45 seconds and then placed in a counting chamber under a microscope. *S. trachea* eggs were identified by their size (80-100 μm × 50-60 μm), shape (bipolar), and visible opercula at both ends. The eggs were then counted by eye, with a handheld Redington Counter.

3.3 TOTAL PLASMA IMMUNOGLOBULIN ELISAS

A direct ELISA was used to determine the concentrations of the immunoglobulins IgY, IgM and IgA in the sparrow plasma samples. The methods were based on Fassbinder-Orth et al. (2016). HRP-conjugated goat anti-chicken antibodies were used for detection. The positive and negative controls were chicken and cow sera, respectively; and a blank containing only coating buffer was included as another control. Three ELISAs were run, one for each immunoglobulin isotype to be detected. The same method was used for all three, but with different plasma dilutions and detection antibody concentrations. The assay was optimised using chequerboard titrations to find which concentration of antigen, plasma, and detection antibody yielded the most easily detectable results.

The plasma samples were diluted (1:10 000 for IgY, 1:1000 for IgM and 1:25 for IgA) in coating buffer (0.015 M Na_2CO_3, 0.035 M $NaHCO_3$, pH 9.6), and 75 µL of each diluted plasma sample was added in duplicate to a flat-bottomed 96-well plate. The plate was then incubated overnight at 4 °C. The next step was to remove the coating solution, then add blocking buffer [PBS (10mM phosphate buffer, 150 mM NaCl, pH 7.4) with 5% non-fat dry milk, 0.05% Tween] and incubate at room temperature for 30 minutes. Then the plate was washed 4 times with 200 µL wash buffer (PBS with 0.05% Tween), using a multi-channel pipette. Next, the detection antibody was added (concentrations were: 1:10 000 for IgY, 1:100 for IgM and 1:1000 for IgA), and the plate was incubated at 37 °C for 1 hour. Then, the washing step was repeated, and TMB-peroxidase substrate (100 µL) was added to each well. The plate was then incubated at room temperature in the dark for 8 minutes before the reaction was stopped with H_2SO_4 (1 M, 100 µL). Then the plate was read immediately at 450 nm with a microplate reader (Cytation 5 Image Reader).

3.4   PARASITE ANTIGEN PREPARATION

S. trachea parasites collected from house sparrows were washed with PBS, then stored at -80 °C. This was done in a previous year. Then they were weighed and homogenised by a pestle homogeniser with 150 µL PBS. After homogenisation, the pestle was rinsed with 100 µL PBS that went into the homogenate. The homogenate was then centrifuged at 10 000 rpm for 15 minutes at 4 °C. The supernatant was collected in an Eppendorf tube. The protein concentration was estimated with the protein A280 assay on a Nanodrop spectrophotometer. Then the extract was diluted to approximately 1 mg/mL protein concentration with PBS and stored at -80 °C.

3.5   *S. TRACHEA* ANTIGEN-SPECIFIC ELISA

To determine the specificity of sparrow IgY to *S. trachea* crude extract antigens, an indirect ELISA was used. The methods were adapted from Fassbinder-Orth et al. (2013). It was not possible to measure the concentration of more than one antigen-specific immunoglobulin isotype because of a limited amount of both parasite material and sparrow plasma. I chose to test for IgY since there was a higher probability of detecting it. This was because of its relatively high concentration in the plasma (as measured in the total Ig ELISA), and because the detection antibody that was used for IgY (goat anti-chicken IgY) had the highest affinity in the total Ig

ELISA. The assay was optimised using chequerboard titrations in the same way as in the total immunoglobulin ELISA.

Parasite homogenate [100 μL, 1:100 in coating buffer (0.015 M Na2CO3, 0.035 M NaHCO3, pH 9.6)] was added to the wells of a 96-well polystyrene plate, then the plate was incubated overnight at 4 °C. The coating buffer was removed, blocking buffer was added to the wells (200 μL, PBS with 5% non-fat dry milk, 0.05% Tween) and the plate was incubated at room temperature for 30 minutes. Then the blocking buffer was removed and sparrow plasma [100 μL, 1:100 in wash buffer (PBS with 0.05% Tween)] was added in duplicate. The plate was incubated at 37 °C for 1 hour. After this, the plate was washed four times with wash buffer (200 μL) and goat anti-chicken IgY detection antibody (100 μL, 1:1000 in blocking buffer) was added before the plate was incubated at 37 °C for 1 hour. The washing step was repeated and TMB (100 μL) was added to the wells. Then the plate was incubated at room temperature for 10 minutes. Finally, the reaction was stopped with H_2SO_4 (100 μL, 1 M) and the plate was read at 450 nm with a microplate reader.

3.6 DATA ANALYSIS

For each successful ELISA plate run (total IgY, total IgM and antigen-specific IgY), two linear mixed effect models were run using R (Appendix B) (R Core Team, 2021) and the R-packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), readxl (Wickham & Bryan, 2019) and dplyr (Wickham et al., 2021). Both models had the relative immunoglobulin concentration (P/N: ELISA value divided by the negative control) as the response variable, age (adult or juvenile) and island (Hestmannøy or Træna) as fixed factors and body condition (calculated as the unstandardized residuals from a regression of body weight on tarsus length, as described by Holand et al. (2015)) as a fixed effect covariate. One model (model 1) had *S. trachea* infection status (infected = birds with FEC > 0 at the time of sampling, or non-infected = birds with FEC 0) as the last fixed factor, while the other (model 2) had FEC (faecal egg count) as a fixed effect covariate. Duplicate ELISA P/N values for each individual were included in the models, so to control for individual repeated measurements, the bird ID of each data point was included as a random factor in both models. Since most juveniles were of unknown sex, the number of birds with known sex was small (19 of a total of 36 birds). Thus, sex was not included as a fixed factor in the models as it would reduce power. Models with an interaction between island and infection status were tested, but are not presented because there was no evidence of any

13

interaction effects. Instead of relying on statistical significance testing with a set p-value threshold, the results are presented in the gradual language of evidence (Muff et al., 2022). Plots were made in R using the packages ggplot2 (Wickham, 2016), tidyr (Wickham, 2021) and ggpubr (Kassambara, 2020).

4 RESULTS

4.1 INFORMATION ABOUT THE SAMPLED BIRDS

The total number of birds sampled was 98, 49 from Hestmannøy and 49 from Træna. However, only 18 of the sampled birds were infected with *S. trachea*, 7 from Hestmannøy and 11 from Træna. All the samples from infected birds were analysed, and 18 non-infected birds were selected as the control group. When selecting the non-infected samples, the main focus was on selecting samples with a large amount of plasma with no blood contamination. They were also selected to keep approximate equal proportions between islands and sexes (Table 4.1). The infected birds, however, were not evenly distributed. Only 4 of the 18 birds were adults. In the control group, 4 of 18 birds were adults as well, to have approximately similar structures in the infected and non-infected samples. Many of the juveniles were of unknown sex, which is why sex was not included in the models.

Table 4.1: Sample size, mean faecal egg count (FEC), number of infected birds, age distribution (adult/juvenile/unknown) and sex distribution (female/male/unknown) of the sample from each island.

Island	Sample size	Mean FEC	No. of infected birds	Age (A/J/U)	Sex (F/M/U)
Hestmannøy	16	10,6	7	6/10/0	7/7/2
Træna	20	67,4	11	1/17/2	2/3/15

4.2 TOTAL IMMUNOGLOBULIN CONCENTRATION IN PLASMA

The total concentration of the immunoglobulins IgY and IgM in the plasma of the sparrows was measured by direct ELISA. IgA was also measured, but its concentration was too low to be detected in the ELISA. For each immunoglobulin, two models were run with immunoglobulin concentration (corrected for background noise) as the response variable. Of the following results, only those concerning FEC are from model 2, the rest are from model 1 (Appendix B). There was no evidence for a difference in total immunoglobulin concentrations between infected and non-infected birds for either IgY (-0.430, SE = 0.529, P = 0.42) or IgM (0.130, SE = 1.14, P = 0.91) (Figure 4.1a). There was no evidence for a relationship between the faecal egg count (FEC) and the total IgY concentration (0.00114, SE = 0.00360, P = 0.76), but moderate evidence of a positive relationship between FEC and the total IgM concentration (0.0131, SE = 0.00598, P = 0.048) (Figure 4.1d). However, this relationship is caused by one

outlier (Figure 4.1d), and without it, there is no evidence of a relationship between FEC and IgM (-0.006, SE = 0.017, P = 0.73). As for differences in total immunoglobulin concentrations between the two islands, there was strong evidence for a lower concentration on Træna, both for IgY (-2.06, SE = 0.563, P = 0.00095) and IgM (-3.96, SE = 1.21, P = 0.0026) (Figure 4.1b). For IgM, there was moderate evidence of a higher concentration in juveniles than adults (3.82, SE = 1.43, P = 0.012), while for IgY, there was only very weak evidence of this (1.08, SE = 0.662, P = 0.11) (Figure 4.1c). As for body condition, there was no evidence of it being related to either total IgY concentration (3.43, SE = 5.34, P = 0.53) or total IgM concentration (-1.24, SE = 11.5, P = 0.91).

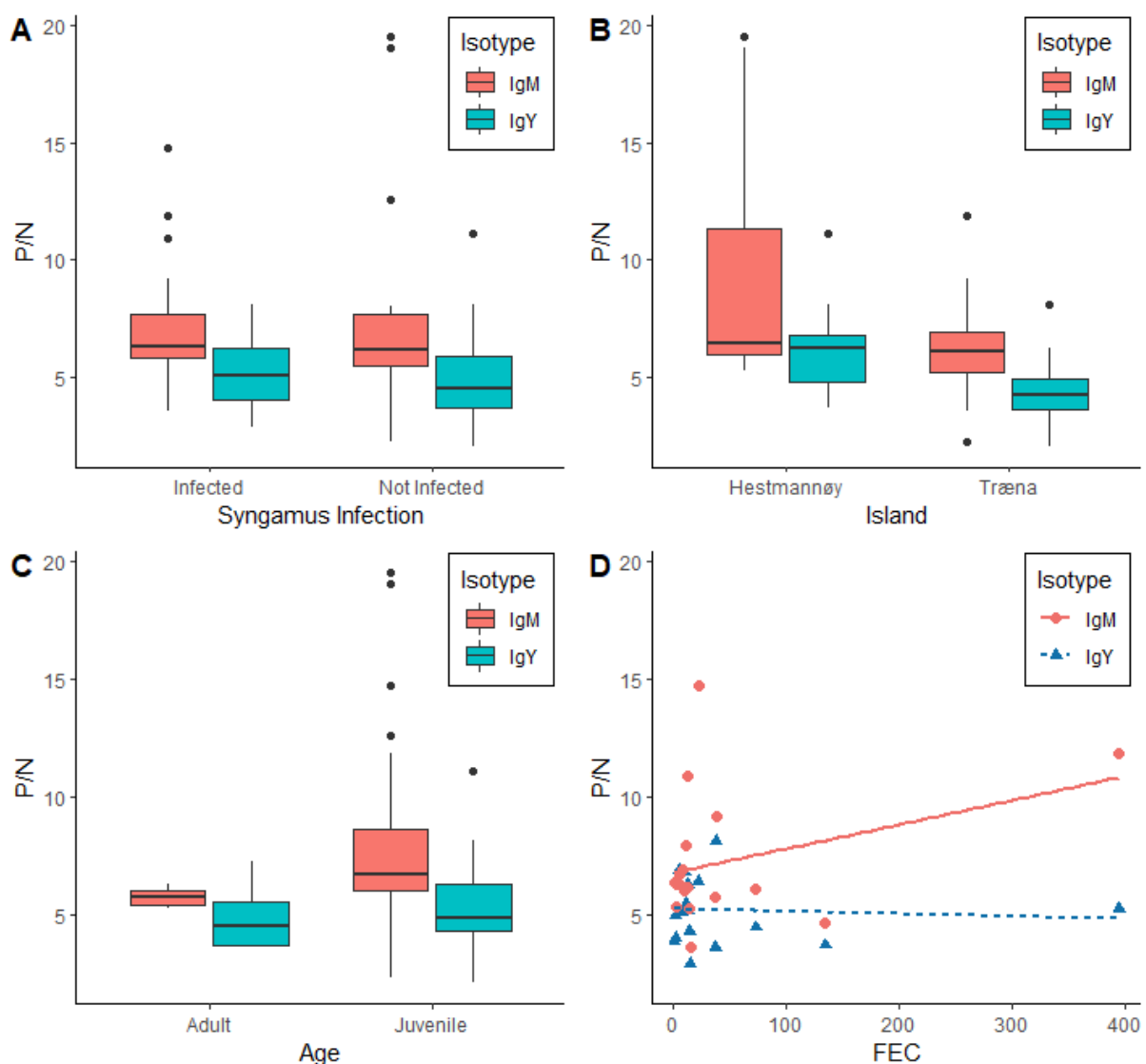**Figure 4.1:** Plots of the raw data showing: (A) Relationship between *S. trachea* infection status and total plasma concentrations of IgY and IgM. (B) Relationship between the island a sample was taken and the total plasma concentrations of IgY and IgM. (C) Relationship between bird age (adult or juvenile) and its total plasma concentration of IgY and IgM. (D) Relationship between faecal egg

count (FEC) and total plasma concentrations of IgY and IgM. P/N signifies positive/negative, meaning the sample ELSIA value (OD) divided by the ELISA value of the negative control.

4.3 *S. TRACHEA* ANTIGEN-SPECIFIC IGY CONCENTRATION IN PLASMA

The plasma concentration of *S. trachea* antigen-specific IgY was measured by indirect ELISA. The following results concerning FEC are from model 2, while the remaining results are from model 1. There was moderate evidence of there being a higher concentration of antigen-specific IgY in birds infected with *S. trachea* than in non-infected birds (1.95, SE = 0.780, P = 0.018) (Figure 4.2a). However, there was no evidence of a relationship between FEC and antigen-specific IgY concentration (-0.00435, SE = 0.00668, P = 0.53) (Figure 4.2d). Nor was there evidence of a difference in antigen-specific IgY concentrations between islands (-0.153, SE = 0.829, P = 0.86) (Figure 4.2b). In contrast, there was very strong evidence of juveniles having a lower plasma concentration of antigen-specific IgY than adults (-5.53, SE = 0.976, P = 3.2 * 10^{-6}) (Figure 4.2c). Lastly, there was no evidence of a relationship between body condition and antigen-specific IgY concentration (6.05, SE = 7.87, P = 0.45).
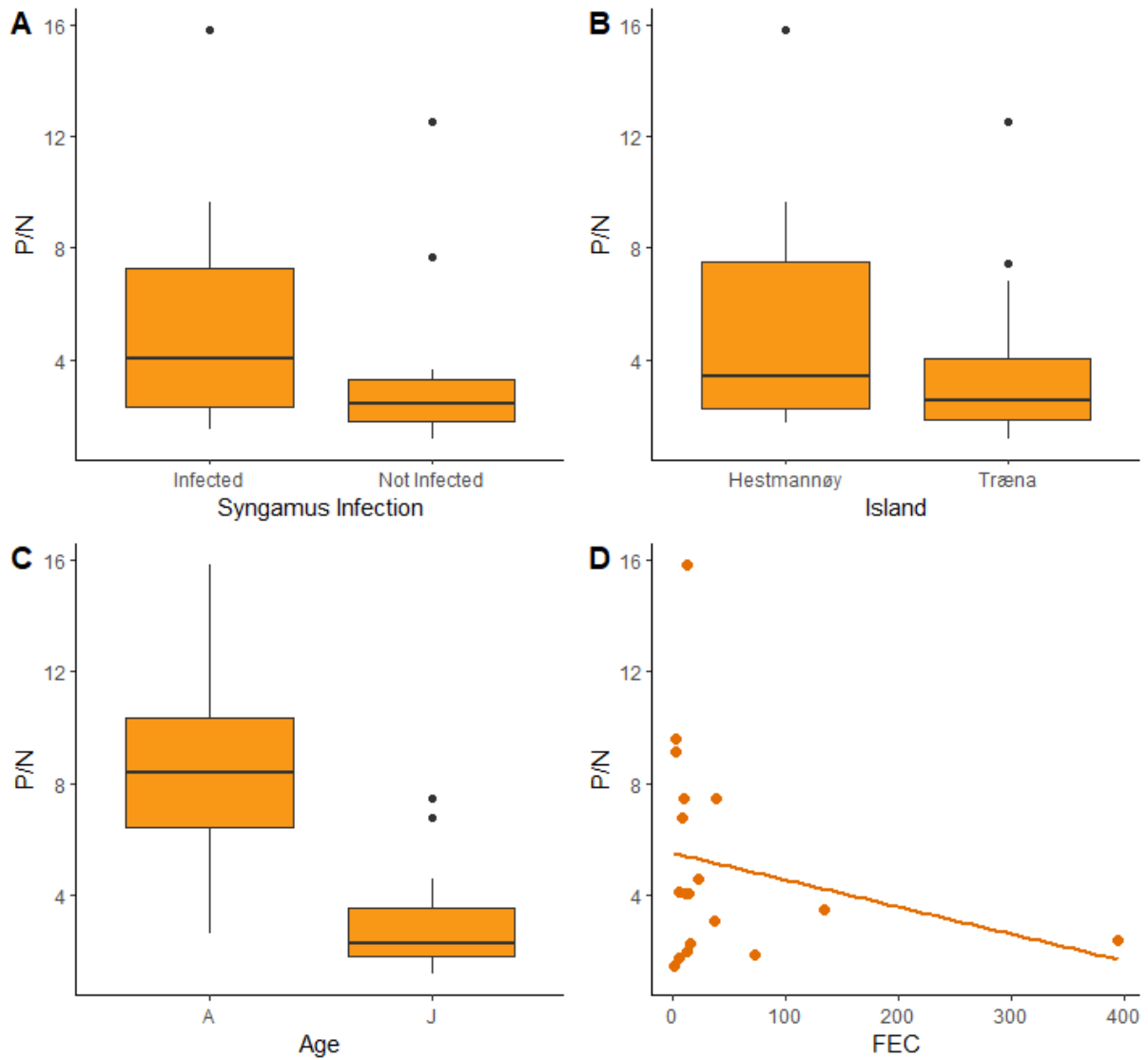
Figure 4.2: Plots of the raw data showing: (A) Relationship between *S. trachea* infection status and plasma concentration of antigen-specific IgY. (B) Relationship between the island a sample was taken on and the plasma concentration of antigen-specific IgY. (C) Relationship between bird age (adult or juvenile) and its plasma concentration of antigen-specific IgY. (D) Relationship between faecal egg count (FEC) and plasma concentrations of antigen-specific IgY. P/N signifies positive/negative, meaning the sample ELSIA value (OD) divided by the ELISA value of the negative control.

5 DISCUSSION

In this study, the levels of different immunoglobulin isotypes in house sparrow plasma were quantified to explore the relationship between parasite load and total plasma immunoglobulin concentration. It was also investigated whether parasite-specific Ig levels were linked to infection status or FEC. The results showed that house sparrows have higher total plasma concentrations of IgY and IgM on Hestmannøy than on Træna, and that the total IgM concentration was higher in juveniles than in adults. Additionally, *S. trachea*-infected house sparrows had a higher plasma concentration of *S. trachea*-specific IgY than non-infected ones, and *S. trachea*-specific IgY levels were significantly higher in adults than in juveniles.

5.1 INTERPRETATION OF THE RESULTS

There was no evidence of a relationship between total immunoglobulin concentration and *S. trachea* infection status for either IgY or IgM. The results of the model showed a moderate positive relationship between total IgM concentration and FEC, but as this result was strongly affected by one outlier (Figure 4.1d), there did not seem to be an actual relationship. Nonetheless, the concentration of nematode-specific IgM has previously been shown to increase during nematode infection in sheep experimentally infected with the parasitic nematode *Trichostrongylus colubriformis* (Pernthaner et al., 2006). The reason we did not see a relationship between FEC and either IgY or IgM could be that sparrows were at different stages of infection, and FEC varies throughout these stages. FEC has a peak at about one week after infection, after which it declines rapidly (Seivwright et al., 2004). Additionally, FEC varies throughout the day (Wongrak et al., 2015), meaning the time of sampling had an effect as well. This makes FEC a noisy measurement in cases when sampled individuals are not infected or sampled at the same time.

There was moderate evidence of a higher concentration of antigen-specific IgY in infected birds. This means that sparrows infected with *S. trachea* have more IgY antibodies specific to *S. trachea* antigens; a result that suggests that the adaptive immune system, and IgY specifically, is involved in the immune response to *S. trachea* in house sparrows. However, there was no evidence of a relationship between antigen-specific IgY concentration and FEC. This, as with total Ig, is probably because the sparrows were at different stages of infection, making FEC a noisy measurement.

The lack of a clear relationship between total immunoglobulin concentrations and parasite load might be caused by the presence of other pathogens, that were not accounted for in this analysis, affecting the total immunoglobulin levels. As there was strong evidence of a difference in total immunoglobulin levels between the two islands but not in the antigen-specific IgY levels, there are evidently other factors that affect the total immunoglobulin concentrations. The relatively high concentration of both total IgY and total IgM on Hestmannøy is likely due to environmental differences. The sparrow population on Hestmannøy lives on farms among livestock, while the Træna population lives in gardens. Farm environments are pathogen-dense due to the presence of high numbers of domestic livestock (de Rooij et al., 2019; McAuley et al., 2014; Oliver et al., 2005), and pathogens can spill over both to wild populations from livestock and vice-versa (Jori et al., 2021; Palmer et al., 2012). As the levels of *S. trachea*-specific IgY did not differ between islands in the present study, but total immunoglobulin levels did; there is likely a difference in the general pathogen levels between the two environments, but no significant difference in *S. trachea* prevalence. However, Holand et al. (2013) found that parasite prevalence varied considerably in both time and space among sparrows in the Helgeland metapopulation. This prompts the question of why there was no evidence of different *S. trachea*-specific IgY concentrations if there was a difference in parasite prevalence between the two islands, when the *S. trachea*-specific IgY concentration is higher in infected birds. It could be that the spatial prevalence pattern has changed since the study by Holand et al. (2013) and that Hestmannøy and Træna had a more similar *S. trachea* prevalence in 2021, during this study.

For IgM and IgY, there was moderate and very weak evidence, respectively, of a higher total immunoglobulin concentration in juveniles than in adults. However, for antigen-specific IgY, there was very strong evidence of a lower concentration in juveniles than adults. This is as expected because the adults have lived longer and may have built up an immunity to *S. trachea* from earlier exposure. IgY is the predominant isotype in secondary antibody responses, so the higher concentration in adults could indicate that the adults have been exposed before (Härtle et al., 2014). Looking at the data, there is moderate evidence for the non-infected adults having a higher concentration of parasite-specific IgY than the non-infected juveniles (5.05, SE = 1.49, P = 0.004), which supports the theory that they have been previously exposed, but the non-infected juveniles have not. Adults have built immunity to different pathogens over time,

resulting in a better immune response among birds that have made it to adulthood (Sol et al., 2003).

The total IgM concentration in juveniles could mean that sparrows have a higher IgM concentration as juveniles, but it could also be a result caused by the low ratio of adults to juveniles in the sample. Even though there is moderate evidence ($P = 0.012$), this is based on only 4 infected and 4 non-infected adults. Nevertheless, the result is in concordance with studies on humans showing that IgM is the first immunoglobulin expressed during foetal development (Van Furth et al., 1965), and in the spotted hyena, where total IgG and IgA levels were higher in juveniles than in adults (Ferreira et al., 2021). However, there is a lack of research into differences in IgM levels between adults and juveniles in wild animals, so this should be further examined with larger sample sizes.

5.2 SIGNIFICANCE OF FINDINGS

One of the most important findings from this study is that *S. trachea*-infected house sparrows have a higher plasma concentration of *S. trachea*-specific IgY than non-infected ones. This means that IgY and the adaptive immune system are involved in the sparrows' immune response to *S. trachea*. The higher concentration in infected birds indicates that *S. trachea* infection triggers an adaptive immune response, and a natural next step would be to further research the genetics of immunoglobulins in birds, particularly IgY. Several loci linked to *S. trachea*-infection have already been identified, some of which are associated with the adaptive immune system, particularly with the MHC (Lundregan et al., 2020). Holand et al. (2013) found that parasite prevalence dynamics differed between sparrow populations on the different islands, suggesting that genetic differences in the immune system may affect the host-parasite relationship in addition to any environmental causes. It would be interesting to look for more genomic regions that are associated with the adaptive immune system now that we know that IgY is involved in the response to *S. trachea*. Regions of interest are those that code for immunoglobulin paratopes, as well as the MHCII since the diversity of MHCII proteins plays an important role in binding to the optimal antigen peptides to present on the cell surface (Duffy et al., 2017; Mikocziova et al., 2021).

Another important finding is the difference in *S. trachea*-specific IgY concentrations between adult and juvenile house sparrows. This finding suggests that adult sparrows have a degree of

acquired immunity to *S. trachea*. Even though the higher level of antigen-specific IgY did not mean that no adults were infected, none of the adults measured in the present study was severely infected (Appendix A, Table A.2). This agrees with the fact that helminth infection rates and the severity of infections are higher in juveniles than adults in several bird species (Cooper & Crites, 1974; Davidson et al., 1977; Slater, 1967). Future studies should look into whether *S. trachea*-specific IgM and IgA are also higher in adults than in juveniles, thus revealing even more of the immune dynamics of house sparrows.

5.3 LIMITATIONS

The biggest limitation of the present study was perhaps the sample size, particularly that of adult infected birds. Because of practical reasons during the sampling (after the taking of a blood sample, the sample had to be centrifuged immediately, then frozen), collection of plasma samples could only be done where there was access to a centrifuge and freezer. Although sampling of birds from both Træna and Hestmannøy allowed us to make important comparisons in total Ig levels between farm and non-farm islands, a mobile generator and freezer setup could allow the collection of plasma samples from birds present on additional islands. A larger sample size would increase power to detect any relationship between Ig levels and FEC and reduce experimental noise.

Another limitation was the blood collection method. From most of the birds, less than 150 μL of blood was collected, as they often did not bleed that much from a puncture in the brachial vein. Because of this, many of the plasma samples collected were small, but in the end, there was enough plasma from all the infected birds. It is uncertain whether there is a better way to collect more plasma that is still safe for the birds. Taking blood from the tarsus is possible, but it is important to not exceed the recommended limit for blood volume extraction. The recommendation for poultry is 1% of the body weight (Kelly & Alworth, 2013). In the present study, the maxim limit was set to 200 μL of blood in a single sampling of one bird, which is approximately 0.7% of the body weight. A couple of the collected plasma samples were contaminated with other blood components because they did not separate completely. This lower degree of separation during centrifuging seemed to happen more frequently when the amount of blood in the capillary tube was small. The contamination was however not likely to have affected the results, as the samples were highly diluted prior to the ELISAs.

22

IgA was not detected in the ELISA, meaning it had a too low affinity to anti-chicken IgA to be detected at the dilutions used. In future, it would be interesting to measure the IgA concentration as it has been linked to parasite infections (de la Chevrotière et al., 2012; Henderson & Stear, 2006; Strain et al., 2002). A higher dilution of either plasma or both plasma and detection antibody may solve the problem of detecting the IgA.

5.4 FURTHER STUDIES

It would be interesting to do an ELISA to measure the concentration of antigen-specific IgM, as that was not done in this study. The results showed no relationship between *S. trachea*-specific IgY and FEC, even though there was a difference in the concentration of specific IgY between infected and non-infected birds. It might be useful to investigate this further with a larger sample size and to link the findings to genetic diversity at relevant loci as well as data on survival and reproductive success. Identifying the genes coding for IgY light and heavy chains means it would be possible to identify genetic variation in these genes, examine how selection is acting on them, and whether they are evolving due to any selection. This would be very interesting, as the connection between *S. trachea*-specific IgY concentration and infection indicates that there could be selection acting on these genes, and that any resulting evolution should in turn affect prevalence and fitness in the house sparrow populations.

5.5 CONCLUSIONS

Based on the finding that there was a higher concentration of *S. trachea*-specific IgY in sparrows infected with the parasite than those not infected, it is likely that the adaptive immune system, and IgY specifically, is involved in the response to *S. trachea* in house sparrows. It was found that sparrows on the islands of Hestmannøy and Træna differed in their total plasma concentrations of IgY and IgM, but not in their concentration of *S. trachea*-specific IgY. This is likely due to a difference in the number of pathogens in the two habitats, one consisting of farms and the other of gardens, and has implications for the spread of pathogens from farm environments to natural populations. Lastly, it was found that juvenile sparrows have a lower concentration of *S. trachea*-specific IgY, but a higher total concentration of IgM than adults. The lower concentration of specific IgY can be explained by the fact that adults have built a resistance to *S. trachea* from previous exposure, while juveniles have not. The higher concentration of total IgM could indicate that juveniles generally have a higher concentration

of IgM, but it could also be because the number of adult birds sampled was much lower than the number of juveniles.

In future, studies should look into whether genetic diversity at Ig loci, especially IgY, is linked to the survival of infection by *Syngamus trachea* and whether the genes coding for these loci are targets of selection. In a world where ecosystems are rapidly altered by human influence, host-parasite interaction is one of the many ecosystem components that we have to monitor. To be able to do this, we have to increase our understanding of molecular and other mechanisms involved in host-parasite interactions.

6 ACKNOWLEDGEMENTS

7 IMAGE CREDITS

Figure 2.1: "Antibody basic unit" (https://commons.wikimedia.org/wiki/File:Antibody_basic_unit.svg) by Tokenzero (https://www.mimuw.edu.pl/~mw290715/) is licenced under CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0/deed.en).

Figure 2.2: "ELISA diagram" (https://commons.wikimedia.org/wiki/File:ELISA_diagram.png) by Cavitri is licenced under CC BY 3.0 (https://creativecommons.org/licenses/by/3.0/deed.en).

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9 APPENDIX A: RAW DATA

Four ELSIAs were run, to measure total IgY (Table A.1a), total IgM (Table A.1b), total IgA (Table A.1c), and *S. trachea* antigen-specific IgY (Table A.1d). Below are tables representing the ELISA microtiter plates, showing the measured OD values of the samples, as well as a table showing the data for individual birds sampled (Table A.2).

Table A.1: Representations of ELISA plates showing OD values of (A) total IgY, (B) total IgM, (C) total IgA, and (D) *S. trachea*-specific IgY in house sparrow plasma measured by spectrophotometry. Each pair of cells next to each other (e. g. A1 and A2) are duplicates of the same sample. White cells represent non-infected birds, orange represent infected, and grey cells are the controls (From top to bottom: blank, chicken plasma, bovine plasma).

A	1	2	3	4	5	6	7	8	9	10	11	12
A	0.212	0.225	0.709	0.612	0.278	0.258	0.389	0.371	0.165	0.179		
B	0.266	0.259	0.275	0.260	0.212	0.222	0.365	0.376	0.216	0.242		
C	0.403	0.381	0.216	0.224	0.359	0.447	0.265	0.263	0.320	0.302		
D	0.268	0.300	0.119	0.125	0.402	0.414	0.324	0.322	0.212	0.212		
E	0.424	0.435	0.199	0.187	0.282	0.307	0.220	0.218	0.058	0.061		
F	0.511	0.452	0.281	0.302	0.307	0.305	0.317	0.285	3.227	3.292		
G	0.305	0.268	0.173	0.166	0.222	0.253	0.484	0.480	0.059	0.065		
H	0.379	0.359	0.261	0.245	0.388	0.359	0.252	0.252				

B	1	2	3	4	5	6	7	8	9	10	11	12
A	0.652	0.662	2.321	2.312	0.870	1.004	1.749	1.746	0.408	0.438		
B	0.748	0.700	0.883	0.800	0.601	0.644	0.719	0.765	0.807	0.697		
C	1.522	1.459	0.656	0.633	0.727	0.692	0.666	0.771	1.452	1.362		
D	0.983	0.918	0.289	0.252	0.752	0.827	0.869	1.004	0.663	0.698		
E	0.736	0.652	0.543	0.531	0.593	0.658	0.508	0.583	0.107	0.130		
F	2.430	2.084	0.818	0.696	0.685	0.769	0.797	0.836	3.619	3.553		
G	0.836	0.800	0.590	0.582	0.681	0.810	1.032	1.142	0.165	0.199		
H	0.715	0.693	0.733	0.729	1.236	1.347	0.588	0.661				

C	1	2	3	4	5	6	7	8	9	10	11	12
A	0.069	0.066	0.077	0.084	0.066	0.066	0.075	0.079	0.078	0.121		
B	0.068	0.063	0.064	0.065	0.063	0.064	0.068	0.066	0.071	0.093		
C	0.074	0.070	0.063	0.062	0.066	0.067	0.069	0.063	0.071	0.088		
D	0.069	0.063	0.060	0.061	0.062	0.063	0.066	0.065	0.067	0.073		

	1	2	3	4	5	6	7	8	9	10
E	0.068	0.065	0.064	0.061	0.064	0.067	0.071	0.070	0.067	0.083
F	0.069	0.065	0.065	0.065	0.064	0.073	0.064	0.064	1.135	1.094
G	0.073	0.065	0.061	0.068	0.076	0.063	0.066	0.067	0.080	0.103
H	0.076	0.070	0.077	0.068	0.072	0.069	0.068	0.075		

D	1	2	3	4	5	6	7	8	9	10
A	0.254	0.247	0.351	0.341	0.175	0.160	0.445	0.430	0.208	0.230
B	0.173	0.156	0.165	0.166	1.305	1.089	0.387	0.396	0.137	0.148
C	0.330	0.301	0.309	0.314	0.681	0.747	0.184	0.179	0.226	0.237
D	0.314	0.282	0.112	0.110	0.172	0.168	0.370	0.404	0.287	0.300
E	0.760	0.705	0.136	0.134	0.895	0.940	0.333	0.333	0.091	0.100
F	0.346	0.322	0.187	0.188	1.423	1.601	0.640	0.655	0.092	0.101
G	0.227	0.197	0.267	0.248	0.871	0.880	0.667	0.758	0.081	0.092
H	0.231	0.202	0.201	0.199	0.191	0.193	0.391	0.383		

Table A.2: Sparrows used in the study and their corresponding data. N.I. = Not Infected, I. = Infected.

Bird ID	Date sampled	Infection status	Island	Sex	Age	FEC	Body condition
8N42931	25. jul	N.I.	Hestmannøy	F	A	0	-0,0842
8P31362	25. jul	N.I.	Hestmannøy		J	0	-0,0842
8917291	27. jul	N.I.	Hestmannøy		J	0	0,0234
8917255	07. aug	N.I.	Hestmannøy	F	J	0	0,0234
8P26017	07. aug	N.I.	Hestmannøy	M	A	0	0,0665
8P31245	08. aug	N.I.	Hestmannøy	M	J	0	0,0665
8917246	08. aug	N.I.	Hestmannøy	M	J	0	0,0544
8P31246	08. aug	N.I.	Hestmannøy	F	J	0	0,0544
8917297	08. aug	N.I.	Hestmannøy	M	J	0	-0,0176
8P31666	28. jul	N.I.	Træna		J	0	-0,0176
8P31911	01. aug	N.I.	Træna	F	A	0	0,0304
8P31852	01. aug	N.I.	Træna		J	0	0,0304
8P31804	01. aug	N.I.	Træna		J	0	-0,0394
8P31915	01. aug	N.I.	Træna	M	J	0	-0,0394
8P26341	01. aug	N.I.	Træna	M	J	0	-0,0176
8P31671	01. aug	N.I.	Træna	F	J	0	-0,0176
8P31721	02. aug	N.I.	Træna		J	0	0,0120
8P26204	04. aug	N.I.	Træna	M	A	0	0,0120
8P31249	08. aug	I.	Hestmannøy	F	A	11	0,0160
8917204	08. aug	I.	Hestmannøy	M	J	6	0,0160
8P31237	07. aug	I.	Hestmannøy	M	A	3	-0,0168
8917263	07. aug	I.	Hestmannøy	F	A	14	-0,0168
8P26099	07. aug	I.	Hestmannøy	F	A	3	0,0104
8P31370	27. jul	I.	Hestmannøy	F	J	14	0,0104
8917293	25. jul	I.	Hestmannøy	M	J	23	-0,0422

8P31503	01. aug	I.	Træna		J	6	-0,0422
8P31851	01. aug	I.	Træna		J	74	0,0458
8P31604	02. aug	I.	Træna		J	12	0,0458
8P31714	02. aug	I.	Træna		J	135	0,1144
8P31723	02. aug	I.	Træna		J	9	0,1144
8P31910	01. aug	I.	Træna	M	J	39	0,0092
8P31605	04. aug	I.	Træna		J	15	0,0092
8P31970	04. aug	I.	Træna		J	16	0,0223
8P31913	01. aug	I.	Træna		J	2	0,0223
8P31696	01. aug	I.	Træna		J	395	-0,0327
8P31972	04. aug	I.	Træna		J	38	-0,0327

10 APPENDIX B: R-MODELS

Linear mixed-effects models (Figure B.1 and B.2) were run in R to determine the relationship between immunoglobulin concentrations and infection status or faecal egg count (FEC).

Figure B.1: Model 1: Linear mixed-effects model for determining the relationship between *S. trachea* infection status and concentrations of total IgY and IgM, as well as parasite-specific IgY.

```
infmodIg <- lmer(P_N ~ S_inf + Age + Body_cond + Island + (1|Bird_ID),
data = Ig, REML = T)
summary(infmodIg)
```

Figure B.2: Model 2: Linear mixed-effects model for determining the relationship between faecal egg count (FEC) and concentrations of total IgY and IgM, as well as parasite-specific IgY.

```
fecmodIg <- lmer(P_N ~ FEC + Age + Body_cond + Island + (1|Bird_ID), data
= Ig, REML = T)
summary(fecmodIg)
```
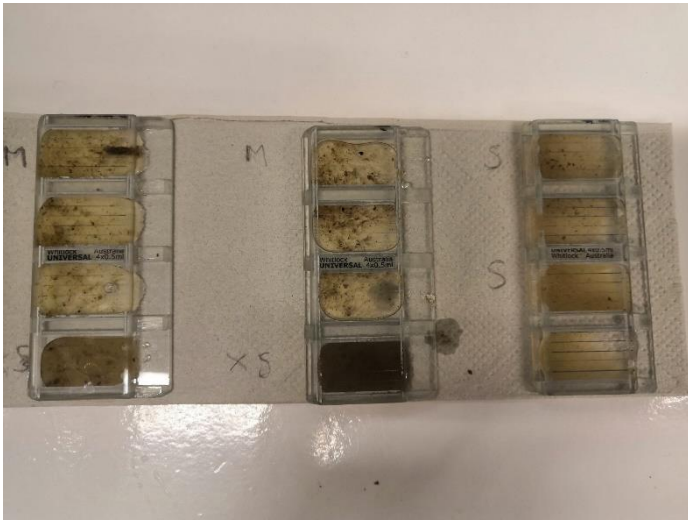
11 APPENDIX C: IMAGES

Figure C.1: Counting chambers containing faecal samples of varying sizes. Medium sized samples were distributed in three chambers, small samples in one or two.
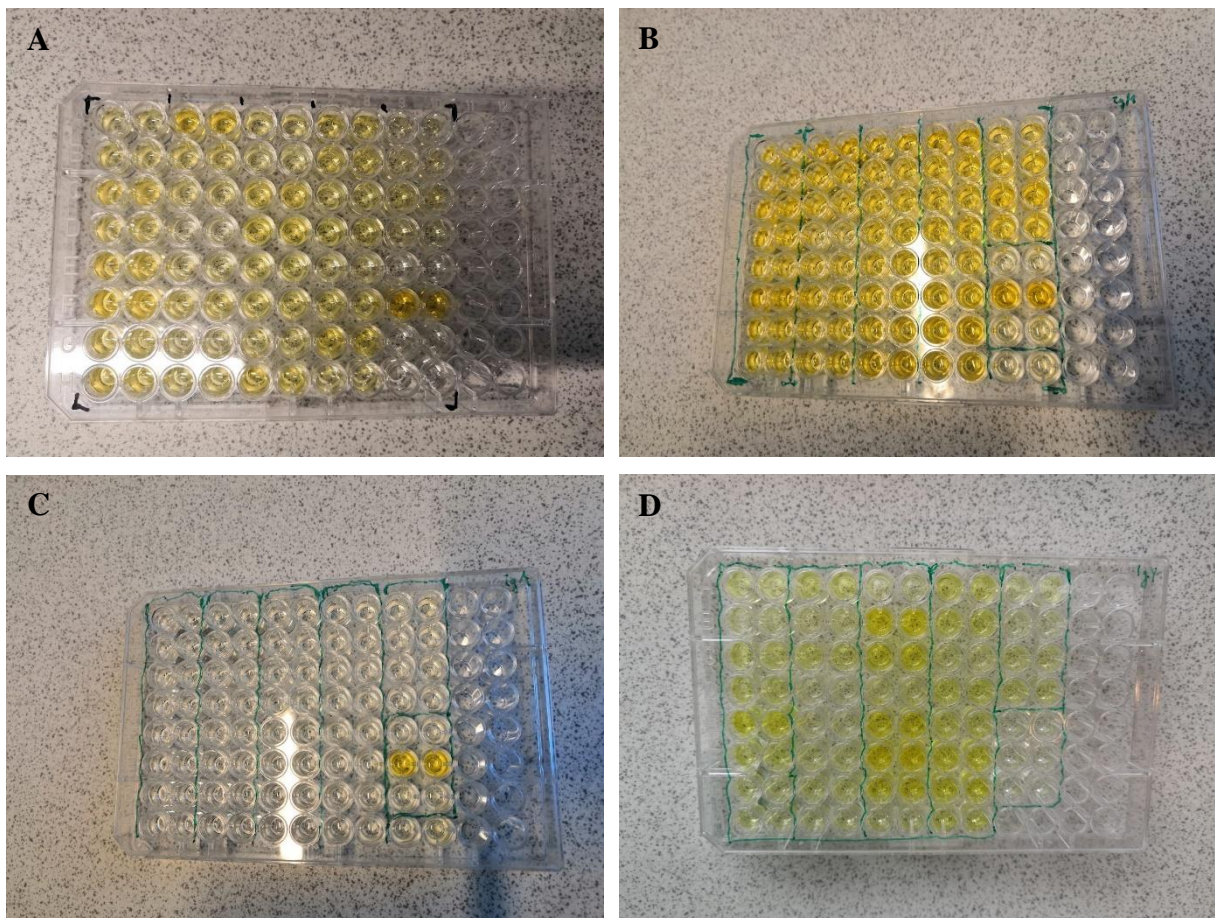

Figure C.2: Microtiter plates after finished ELISA runs for (A) total IgY, (B) total IgM, (C) total IgA, and (D) *S. trachea* antigen-specific IgY. The total IgA ELISA showed no concentration of IgA above background level.