4 Material and methods

4.1 Field studies

In September 2018, wild *Mus musculus* were caught in Brandenburg (permission number: 2347/35/2014). The mice were caught in live traps, which were endued with a mixture of oatmeal and sardines. With the permission of the owners, the traps were positioned at barns and other farm facilities and fetched the next day. The GPS coordinates of each spot, where a trap was positioned, was noted. The caught mice were brought to the field laboratory, where they were put into individual cages and supplied with food and water ad libitum. The mice were euthanised by cervical dislocation no longer than 24 hours after they arrived at the field laboratory. Before the dissection, the mice were weighed and the body and tail size were measured. Among other organs, the caecum was dissected and stored at −80°C.

4.2 Detection of an *Eimeria* infection

4.2.1 Spin column-based nucleic acid purification

DNA extraction

DNA is located in the nucleus of each cell. To extract the DNA from tissue, the cells have to get lysed, the nuclear envelope has to be broken up and everything, except the pure DNA, has to be sorted out. DNA extraction was done from caecum tissue by using the „InnuPREP DNA Mini Kit “[1], following the optimized protocol [11]. During the extraction process, the tissues were stored on ice. The extracted DNA was stored at −20°C to avoid degradation.

RNA extraction

Ribonucleic acid (RNA) is the product of the transcription from DNA. RNA implements the genetic information from the DNA to the protein biosynthesis. RNA is, unlike DNA, single stranded, and so prone to degredate faster than DNA. Hence, samples intended for RNA extraction were stored at −80°C. The Kit „PureLink^{TM} RNA Mini Kit“[2] was used for the extraction of RNA from caecum tissue, following the optimized protocol (appendix xy). Test tubes and reagents were kept on ice during the procedure. The extracted RNA was either stored at −80°C or used immediately for reverse transcription.

[1] Analytik Jena AG, Konrad-Zuse-Straße 1, 07745 Jena, Germany
[2] Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008, USA

4.2.2 Nucleic acid quantification

The sample concentration and contamination were measured by using the „NanoDrop 2000/2000c spectrophotometer“[3]. The spectrophotometer was calibrated using the same elution buffer from the respective DNA and RNA extraction protocols. Therefore, 1 μl of the buffer was pipetted on the measurement pedestal and the spectral measurement was initiated. After this, 1 μl of the sample was measured, as the elution buffer before. The signal of the buffer was deducted from the measurements of the sample. Thus only the transmitted signal of the nucleic acid and contamination of the sample was processed.
Nucleic acids have a absorption maximum at 260 nm. The aromatic acids tryptophan and tyrosin have a absoption maximum at 280 nm. Hence, a protein with average amounts of these amino acids has a absorption about 280 nm. The ratio of absorbance 260/280 nm is used to assess a protein contamination of the nucleic acid solution [27]. A ratio of 1.8 is considered as pure DNA, a ratio of 2.0 as pure RNA[28].

4.2.3 Assessment of *Eimeria* infection intensity

For the assessment of the infection intensity, a quantitative polymerase chain reaction (qPCR) was performed. This method monitors the amplification progress of a targeted gene product after every cycle by the use of flourescense signal. SYBR Green 1 was used for monitoring the fluorescense signal, which has a high affinity to bind double stranded qPCR products and a low affinity at single stranded DNA or RNA. The resulting complex emits green light at about 524 nm [29]. SYBR Green 1 binds at primer-dimers and pcr-byproducts as well. As a consequence random products are generated. The SYBR-Green method has a high sensitivity, but low specifity [30].
To estimate the relative presence of parasitic DNA, a sequence of the cell division control protein 42 (CDC42) of the *Mus musculus* nuclear genome was used as a relative quantification reference gene. CDC42 is involved in the regulation of the cell cycle.
To amplify *Eimeria* DNA, a sequence from the mitochondrial cytochrome c oxidase subunit 1 (CO1) was used, which is involved in the aerobic metabolism. The sequences of the primers are listed in table 4.1.

Table 4.1: **Table of qPCR primer.**[11]The CO1 gene is a sequence of the mitochondrial genome of *Eimeria* DNA and CDC42 from the nuclear genome of *Mus musculus*. Both primer pairs amplify a fragment of 200bp. The forward and reverse sequences are shown using the IUPAC code from 5’ to 3’ -end. The amplicon size is shown in bp.

Genome	Target gene	Primer	Sequence (Amplicon size in bp)
mitochondrial	CO1	Eim_COI_qX_F	TGTCTATTCACTTGGGCTATTGT (200)
		Eim_COI_qX_R	GGATCACCGTTAAATGAGGCA
nuclear	CDC42	Ms_gDNA_CDC42_F	CTCTCCTCCCCTCTGTCTTG (200)
		Ms_gDNA_CDC42_R	TCCTTTTGGGTTGAGTTTCC

[3]Thermo Fisher Scientific, 3411 Silverside Road, Bancroft Building, Suite 100, Wilmington, DE 19810, USA

When the fluorescence signal of a target gene passed a set threshold, the cycle number was recorded as the C_T, the cycle threshold number. To generate comparable results, the DNA extracts were standardised to a concentration of 50 $\frac{ng}{\mu l}$. The optimized protocol from Jarquin(2019)[11] was used to perform the qPCR. All sample measurements were arranged in triplicates. The arithmetic mean was calculated out of the three detected C_T values of each sample and target and defined as $C_{T(target)}$. The infection intensity $\Delta C_{T(Eimeria)}$ was calculated with formula 4.1:

$$\text{Infection intensity: } \Delta C_{T(Eimeria)} = C_{T(CDC42)} - C_{T(CO1)} \tag{4.1}$$

The higher the $\Delta C_{T(Eimeria)}$, the higher the realtive prevalence of *Eimeria* DNA in the tissue, thus, a higher infection intensity.

The melting curve was calculated as the negative derivation of the fluorescence intensity and the temperature in percentage. The melting temperature is the maxima of the melting curve, which presents the point of degradation where fifty percent of the DNA is single strand and fifty percent are double stranded.
The melting curve, as well as the melting temperature, depends on the guanine-cytosine content, length and sequence of the qPCR product. Thus, qPCR products can be distinguished by their melting curves [31]. To distinguish random product from real qPCR products, a threshold was set to 33 %. Fluorescence signals above the threshold and at the specific melting temperature were classified as real qPCR products.

An *Eimeria* infection was determined with a infection intensity ($\Delta C_{T(Eimeria)}$) above -5 or with a real *Eimeria* qPCR product in the melting curve.

4.3 Identification of the *Eimeria* subspecies

4.3.1 Polymerase chain reaction

To identify the *Eimeria* subspecies, which were present in the infected caecum, three different genes of three different genomes were amplified by conducting a polymerase chain reaction (PCR). The sequences are listed in table 4.2.
A different sequence of CO1, than it was used for qPCR, was applied as a marker of eukaryotes at species level. Hence the different *Eimeria* subspecies could be differentiated by this sequence. This CO1 primer amplified a fragment of 700 bp and were designed based on the mitochondrial genome of *Eimeria falciformis* [32]. Samples which could not be amplified, were processed with the CO1 primer, which was used for qPCR.
A sequence of the eucaroyotic ribosomal subunit 18s rRNA (18s) in the nuclear genome was used for indentifications at genus level. The primer amplified a fragment of 1500bp and was desingned by Kvicerova [33].
The open read frame 470 (ORF 470) from the apicoplast genome was used to confirm the results of CO1 and 18s. A apicoplast is a plastid, which is found in most *Apicomplexa*, such as *Eimeria* and hosts metabolic pathways[34]. The primer amplified a region of 800bp[35].
To perform these PCRs, the respective protocol for each primer pair was followed(Appendix XY) by using the standardised DNA samples from qPCR. The PCR products were stored at $-20°$C.

Table 4.2: **Table of PCR primer.** Three different target genes from three genomes were used to identify *Eimeria* subspecies. The forward and reverse sequences are shown using the IUPAC code from 5' to 3'-end. The amplicon size is shown in bp.

Genome	Target gene	Primer	Sequence (Amplicon size in bp)
mitochondrial	CO1	Eim_COI_M_F Eim_COI_M_R	ATGTCACTNTCTCCAACCTCAGT (700)[32] GAGCAACATCAANAGCAGTGT
nuclear	18s	Api_18s_kvic_F Api_18s_kvic_R	GAAACTGCGAATGGCTCATT (1500)[33] CTTGCGCCTACTAGGCATTC
apicoplast	ORF 470	ORF470_F ORF470_R	GATGATATATCTTATTATTCAATTCCTT (800)[35] TCCAATATGTAACATTTTATTTCC

Gel-electrophoresis

To verify, whether the PCR was successful and the expected products were amplified, a gel electrophoresis was conducted. A agarose gel was prepared and done as written in XY. The PCR products were mixed with a „DNA Gel Loading Dye (6X)“[4] and were loaded into the gel. The „GeneRuler 1 kb DNA Ladder“[5] was loaded into the first band of the gel as a reference size. The setup was performed with a electrophoresis „EPS 200“[6]. The gel was visualized by a „Molecular Imager® Gel Doc™ XR System“[7].

4.3.2 Subspecies identification

For the samples, which showed a sucessful amplification in the gel, the remain PCR product was purified by following the instructions of the „SAP-Exo Kit“[8]. The purified PCR products with the respective forward and reverse primer were sent to LGC Genomics [9] for sanger sequencing.
The output data was fitted to eliminate low quality regions of the sequence result and the respective forward and reverse sequences were aligned by using the software „Geneious version 6.1.8 “.
To ascertain the *Eimeria* subspecies of the edited sequences, the BLAST (basic local alignment search tool) algorithm was used. A consensus sequence was searched in a database based on the similarity of the sequences[36].

[4]Thermo Fisher Scientific, 3411 Silverside Road, Bancroft Building, Suite 100, Wilmington, DE 19810, USA
[5]Thermo Fisher Scientific, 3411 Silverside Road, Bancroft Building, Suite 100, Wilmington, DE 19810, USA
[6]Amersham Pharmacia Biotech, taken over by GE Healthcare, 41 Farnsworth Street, Boston, MA 02210, USA
[7]Bio-Rad Laboratories, Inc.,2000 Alfred Nobel Drive, Hercules, CA 94547, USA
[8]Jena Bioscience, Löbstedter Str. 71, 07749 Jena, Germany
[9]LGC Genomics GmbH, Ostendstr. 25, 12459 Berlin, Germany

4.4 Detection of the immune response

4.4.1 Reverse transcription

Reverse transcription is the conversion from a RNA template into a DNA single strand, so called complementary DNA (cDNA). For detecting the protein expression level of a cell, the messenger RNA (mRNA) is used, since it contains the protein sequences. During the translation, the mRNA gets polyadenylated at the 3' end[37]. An oligo-desoxythymidine primer can bind to this poly-adenosin tail and recruits the reverse trancriptase. Thus, only mRNA from the RNA extract is converted. The cDNA product is more stabil than RNA and can be used for qPCR analsysis.
The reaction package „iScript^{TM} cDNA Synthesis Kit“ [10] was used to process the reverse transcription. Based on the manufacture protocol, the optimal synthesis efficiency can be achieved using 1μg of RNA. Therefore, the needed RNA volume was calculated out of the nucleic acid quantification results and used as the RNA template for cDNA synthesis. The reverse transcription was processed by following the manufacture protocol.

4.4.2 Immune gene expression measurements

The immune genes were detected by processing a qPCR with cDNA as template, so called reverse transcriptase quantitaitve polymerase reaction (RTqPCR). To compare the immune gene profile of infected and non infected mice, two groups were created. One group with all infected mice and another, randomly chosen, with the same number of non infected mice.
For this measurement, housekeeping genes (HKG) of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β-Actin were used as a relative quantification reference.
For detecting the immune gene expression, sequences of interleukin 6 (IL-6), interleukin 12 (IL-12rb1) and chemokine receptor 3 (CXCR3) were used.
For detecting specially cell autonomous defence genes, sequences of guanylate-binding protein 2 (GBP2) and immunity related guanosine triphosphatases 6 (IRGA 6) were amplified. The sequences of all targets are listed in table 4.3.

To generate comparable results, the cDNA was standardised to a concentration of 300 $\frac{ng}{\mu l}$. The RTqPCR was conducted as shown in XY. The samples were arranged in triplicates. The $C_{T(target)}$ was calculated as described in 4.2.3.
The geometric mean was calculated out of the two HKGs to create a reference index:

$$\text{Index: } \Delta C_{T(HKG)} = \sqrt{(C_{T(GAPDH)} * C_{T(\beta-Actin)})} \tag{4.2}$$

The respective immune gene expression was calculated by subtracting the $C_{T(target)}$ of each target from the index:

$$\text{Immune gene expression: } \Delta C_{T(expression)} = \Delta C_{T(HKG)} - C_{T(target)} \tag{4.3}$$

[10] Bio-Rad Laboratories, Inc.,2000 Alfred Nobel Drive, Hercules, CA 94547, USA

Table 4.3: **Table of RT-qPCR primer.** The sequences of the two HKG, the three immune genes and the two cell autonomous defence genes are listed. The forward and reverse sequences are shown using the IUPAC code from 5' to 3'-end. The amplicon size is shown in bp.

Type	Target gene	Primer	Sequence (Amplicon size in bp)
Housekeeping genes	GAPDH	M. m. GAPDH F	CATTTCCTGGTATGACAATGAATACG (90)[[38]]
		M. m. GAPDH R	TCCAGGGTTTCTTACTCCTTGGA
	β -Actin	M. m. β-Actin F	CGGTTCCGATGCCCTGAGGCTCTT(100)[[38]]
		M. m. β-Actin R	CGTCACACTTCATGATGGAATTGA
Immune genes	IL-6	M. m. IL-6 F	TAGTCCTTCCTACCCCAATTTCC(76)[[26]]
		M. m. IL-6 R	TTGGTCCTTAGCCACTCCTTC
	IL-12rb1	M. m. IL-12rb1 F	ATGGCTGCTGCGTTGAGAA(108)[[26]]
		M. m. IL-12rb1 R	AGCACTCATAGTCTGTCTTGGA
	CXCR3	M. m. CXCR3 F	GCCTTCCTGCTGGCTTGTAT(246)[[39]]
		M. m. CXCR3 R	TAGCTGCAGTACACGCAGAG
Cell autonomus defence genes	GBP2	M. m. GBP2 F	CTGCACTATGTGACGGAGCTA(115)[[40]]
		M. m. GBP2 R	GAGTCCACACAAAGGTTGGAAA
	IRGA6	M. m. IRGA6 F	GGATCAGGGAAGTCCAGCTT(490)[[21]]
		M. m. IRGA6 R	CGGGGAAGTCATAGTGACAA

4.5 Bioinformatic analysis

All analysis were performed in R version 3.6.0. (R core Team 2019).

4.5.1 Analysis of variance (ANOVA)

A linear model was used to analyse the linear regression of the immune gene expression ($\Delta C_{T(expression)}$) over the infection intensity ($\Delta C_{T(Eimeria)}$) for all infected samples. The output of the function `lm()` gives a descriptive model of the dataset, among others, the p-value of a two sided t-test `Pr(>|t|))`. The significance of variation was tested by using a one-way ANOVA with the function `anova(mod,...)`,using the argument `mod=lm`. The output of this function contains, among other parameters, the probability of the F-test `(PR(>F))`. The null hypothesis of the F-test, that the slope of the linear regression is equal to zero, is rejected, if this probability `(PR(>F))` is less than or equal to the significance level(α)[41]. The significance levels are: `0.05 -> 0.01 *; 0.01 -> 0.001 **; 0.001 -> 0 ***`.

4.5.2 Tukey Honest Significant Differences

The differences of the immune gene expression, between the non infected and infected samples was analysed, by group them by their *Eimeria* species presence. By comparing variances between several groups, an approach is needed, where the α-Niveau is adjusted in every comparing step. By comparing the groups without adjusting the α-Niveau, there is a high risk for continuing type 1 ("false-positive") errors after every comparison. For this reason, Tukey's range test was used, which calculates confidence intervals, simultaneously, after every pairwise comparison. The function `TukeyHSD()` needs an object,

created by `aov()`, which is a fit of a variance model. The output of the Tukey-function is, among others, the `(PR(>F))` which is the adjusted p-value for each group-comparison[41].
The significance levels are: `0.05 -> 0.01 *; 0.01 -> 0.001 **; 0.001 -> 0 ***`.

4.5.3 Non metric multidimensional scaling

To differentiate the non infected samples and infected samples, based on their immune gene expressions, a non metric multidimensional scaling (nMDS) was conducted. This compars several metrics among several dimensions, therefore for this application 48 samples among 5 targets, so 5 Dimensions. NMDS is used for reduction of dimensions, so to analyse and describe the differences of these 5 dimensions of all samples in 2 dimensions. The challenge of nMDS, is to calculate and develop a configuration in the smallest number of dimensions, in which the data can be reconstructed [42]. A stress-value is used, to estimate how well the configuration matches the data [43]. For performing the nMDS with R, a distance matrix was calculated with `dist()` and commited into `isoMDS()` of the package `MASS`(version 7.3-51.4). The function `isoMDS()` calculates the isotonic regression of the distance matrix to fit ideal distances to preserve relative dissimilarity order[44].

5 Results

5.1 Infection presence and intensities

The DNA was extracted from the caecum of 156 wild *Mus musculus* as discribed in 4.2.1. The yield and quality of the extracted DNA was measured as described in 4.2.2. Figure 5.1 illustrates the 260/280 value over the DNA concentration of all samples. It illustrates, that the higher the concentration of the DNA extract, the higher the similarity of the 260/280 ratio. DNA extracts with lower concentrations show more variability in their 260/280 ratios. The mean concentration of extracted DNA is 356.32 $\frac{ng}{\mu l}$. The mean value of the 260/280 ratio is 1.99.

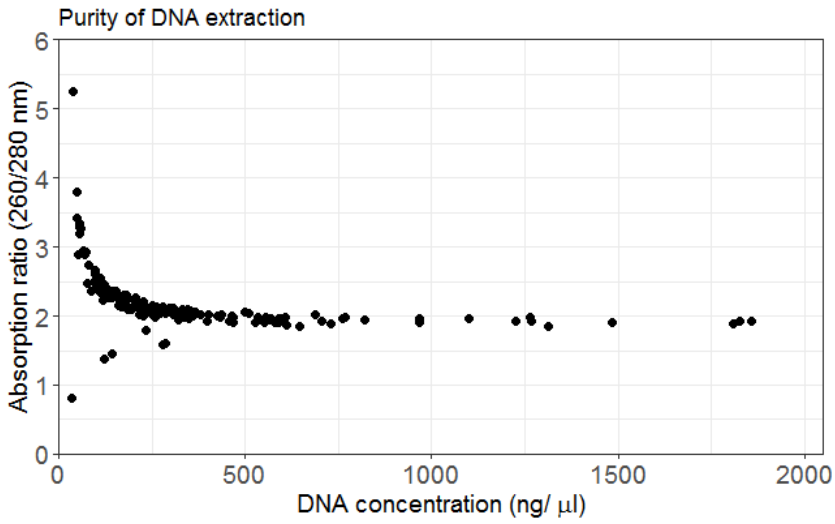


Figure 5.1: **Purity of DNA extraction.** The 156 DNA extracts show a mean purity of 1.99. The plot illustrates, the higher the concentration, the higher the similarity of the 260/280 value. The DNA extracts below 200 $\frac{ng}{\mu l}$ show variability in their 260/280 value.

The qPCR was done as described in 4.2.3. The infection intensity ($\Delta C_{T(Eimeria)}$) was calculated for every sample with formula 4.1. Figure 5.2 shows the fluorescence intensity of two detected samples over the cycle numbers. The green coloured lines illustrate the amplification progress of the *Mus musculus* gene CDC42 and the blue coloured lines the amplification progress of the *Eimeria* gene CO1. The light blue and light green lines belong to sample 601 and the dark green and dark blue lines to sample 554. The C_T value is listed in table 5.1. The infection intensities were calculated and are listed in table 5.1 as well.

The melting curves of sample 601 and 554 are shown in figure 5.3. The negative derivation of the fluorescence intensity of the temperature is plotted over the melting temperature. The melting temperatures are shown in table 5.1.

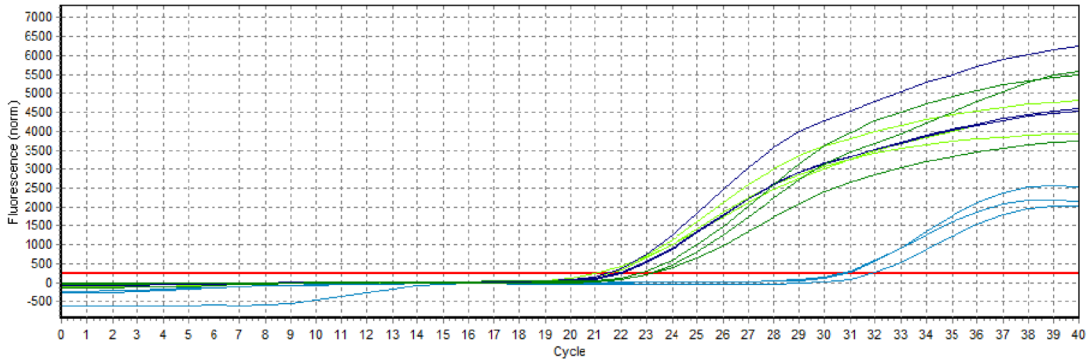


Figure 5.2: **Quantification plot from qPCR.**Exemplary results of sample 601 and 554, illustrated as a quantification plot. The fluorescence intensity is plotted over the cycle number. The threshold is shown as a red line. The blue lines represent the gene CO1 and the green the gene CDC42. The light green and light blue lines belong to sample 601. The dark green and dark blue lines to sample 554.

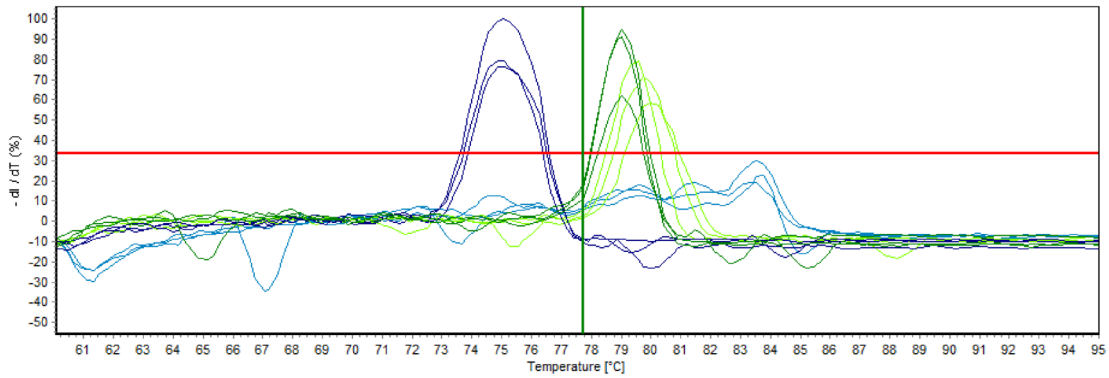


Figure 5.3: **Melting curve from qPCR.** Exemplary results of sample 601 and 554, illustrated as a melting curve. The negative derivation of fluorescence intensity of temperature in percent is plotted over the melting temperature. The setted threshold is shown as a red line. The blue lines represent the gene CO1 and the green the gene CDC42. The light green and light blue lines belong to sample 601. The dark green and dark blue lines to sample 554.

Table 5.1: **Infection intensity analysis.** The C_T values of sample 601 and 554 with their respective arithmetic means ($C_{T(target)}$) according to their targets are listed. The infection intensities ($\Delta C_{T(Eimeria)}$) were calculated. The melting temperatures are listed for the respective targets.

Sample	C_T	$C_{T(target)}$	Target	$\Delta C_{T(Eimeria)}$	melting temperature
601	31,96 30,85 30,70	31,17	CO1	-9,72	84°C
	21,17 21,57 21,61	21,45	CDC42		79°C
554	22,06 21,60 21,94	21,87	CO1	1,25	75°C
	23,18 22,80 23,38	23,12	CDC42		79,76°C

The infection intensity of sample 601 is -9,72 and for sample 554 it is 1,25. All melting curves of 554 show a real *Eimeria* PCR product at the same melting temperature 75 C°. Sample 601 shows a random product under the threshold at 84°C and no *Eimeria* product was amplified. Based on the criteria which are considered in 4.2.3, sample 554 is classified as *Eimeria* infected and sample 601 as non infected.
Out of the analysis for all samples,shows in 24 samples (15,38%) a real *Eimeria* product and are classified as infected.

5.2 Different infection intensities based on *Eimeria* species

The infected samples were analysed as described in 4.3. Eight samples were infected with *Eimeria falciformis* and ten samples with *Eimeria ferrisi*. For six samples, the *Eimeria* genotype could not be determined with the PCR method.
Figure 5.4 shows all 156 samples, classified in TRUE and FALSE for *Eimeria* presence, along there calculated infection intensities (formula 4.1).
The non infected samples are illustrated with red dots. The infected samples are divided into three groups: infetced with *Eimeria ferrisi* (blue), with *Eimeria falciformis* (green) and samples, with no determined genotype, but detected *Eimeria* DNA in the tissue, are labeld as *Eimeria sp.* (yellow).
The samples, which are infected with *Eimeria ferrisi* have an average infection intensity of 0,084. The *Eimeria falciformis* infected samples have an average infection intensity of -3,84 and the *Eimeria sp.* samples have an average infection intensity of -7,14.
Figure 5.4 shows a higher infection intensity for *Eimeria ferrisi* infected samples, than for *Eimeria falciformis* infected samples. Samples of the group *Eimeria sp.* have the least infection intensity and are borderline to the non infected group.

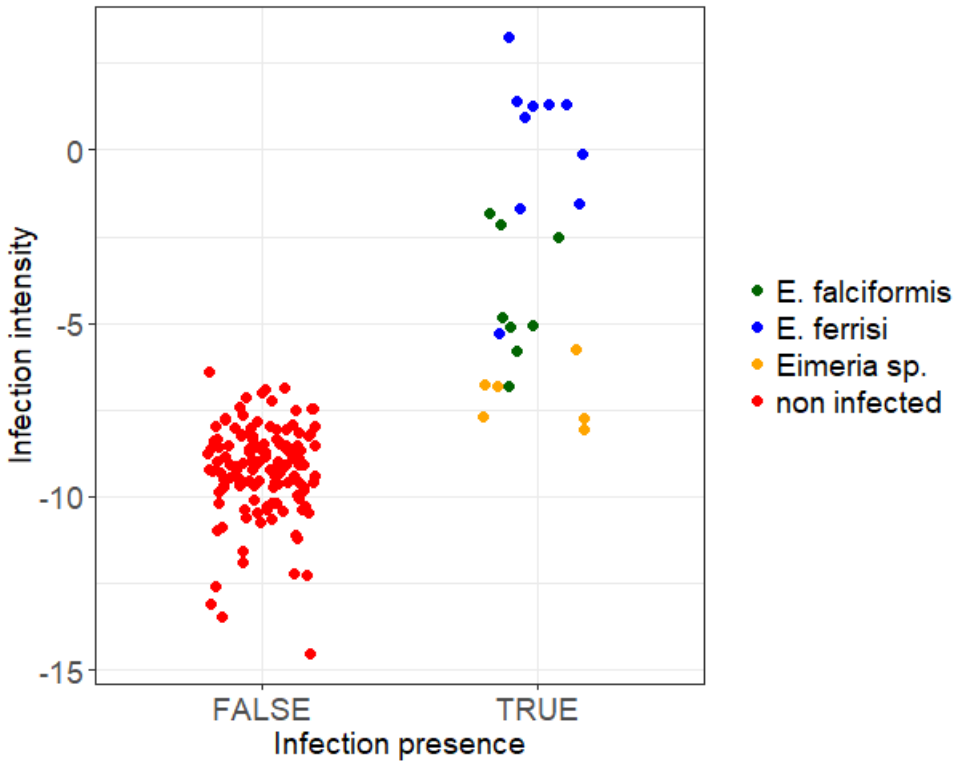


Figure 5.4: **Distribution of the infection intensity.** All 156 samples are classified in infected and non infected and plotted over their infection intensity (formula 4.1). The red dots illustrates all non-infected, blue for *Eimeria ferrisi*, green for *Eimeria falciformis* and yellow for known infections with unknown genotype.

5.3 Immune gene expression

5.3.1 Synthesis of mRNA templates

For the analysis of the immune gene expression two groups were made, as described in 4.4.2. The RNA was extracted from these 48 samples. The average RNA concentration was 1256,57 $\frac{ng}{\mu l}$ and the average 260/280 ratio 1,93. Figure 5.6 illustrates the purity of the RNA extracts.

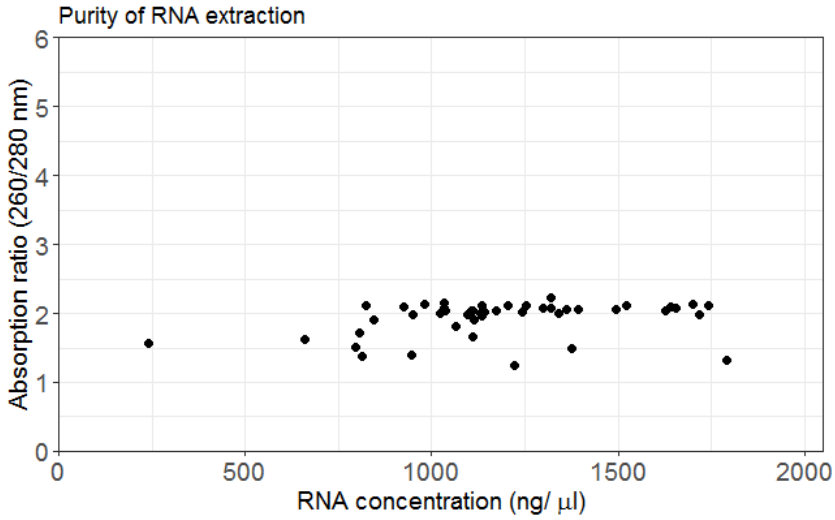
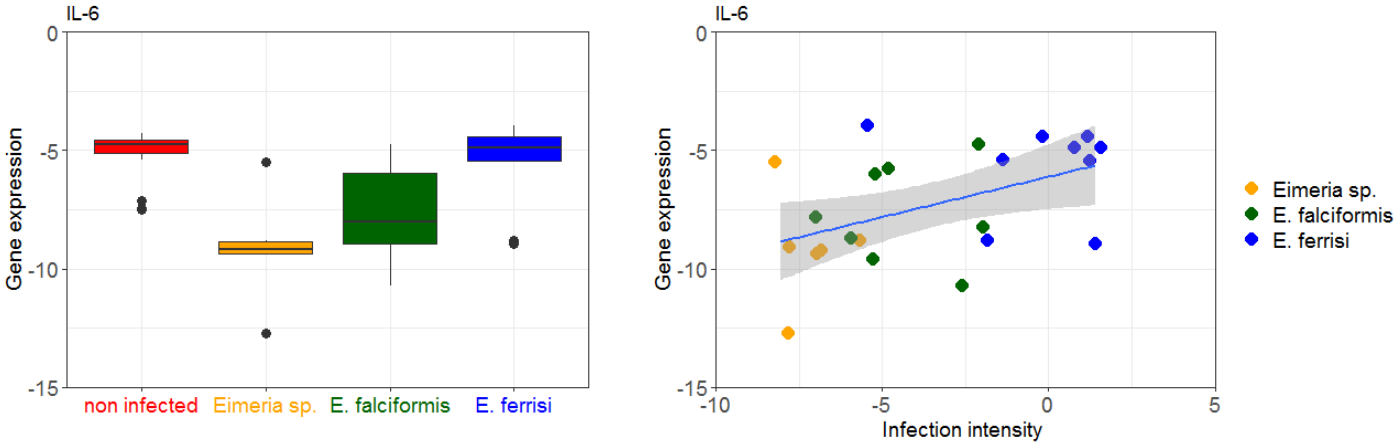


Figure 5.5: **Purity of extracted RNA.** The 48 RNA exctract have an average 260/280 ratio of 1,93 and an average concentration 1256,57 $\frac{ng}{\mu l}$.

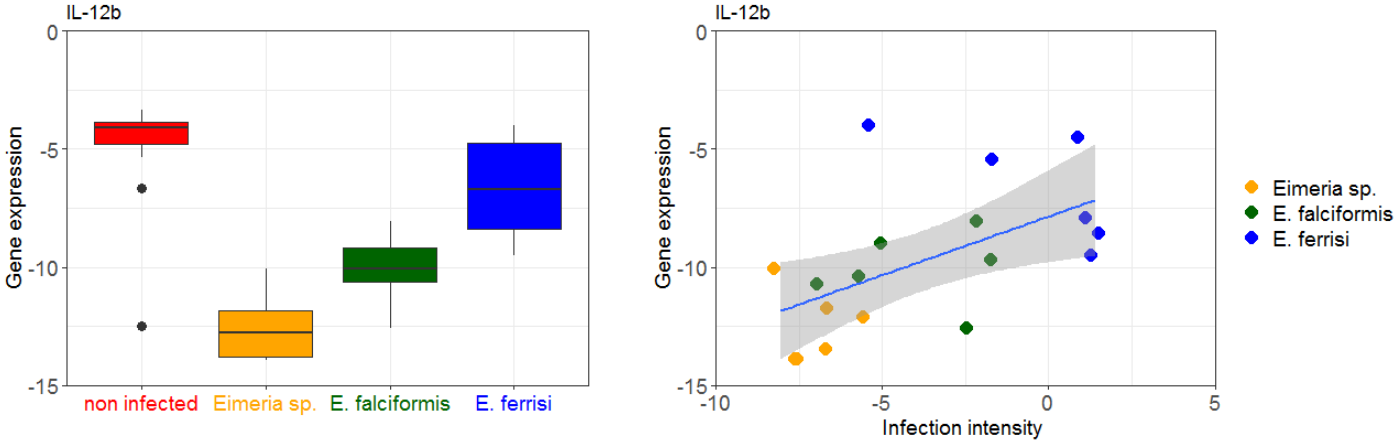
5.3.2 Immune gene expression profiles

The reverse transcription was processed and the needed RNA template was calculated as described in 4.4.1. The RTqPCR was processed as described in 4.2.2. The C_T values were read out from the quantification plot as described in 5.1. The index $\Delta C_{T(HKG)}$ was calculated with formula 4.2 and the immune gene expression $\Delta C_{T(expression)}$ with formula 4.3 for each sample of each target.
To compare the immune gene expression of all infected samples, the gene expression (formula 4.3) for the respective target of all infected samples were plotted over the infection intensity (formula 4.1). A linear model (`lm()`) was fitted and tested, as described in 4.5.1 and illustrated with a blue line. The samples are coloured by their infection status as in figure 5.4.
The immune gene expression of the four different infection types are compared by creating four box plots, according to the colour pattern from figure 5.4. The significant variance between each groups was tested with `TukeyHSD()` as discribed in 4.5.2.
Figures 5.6 to 5.10 show the discribed linear models and boxplots according the respective immune gene.



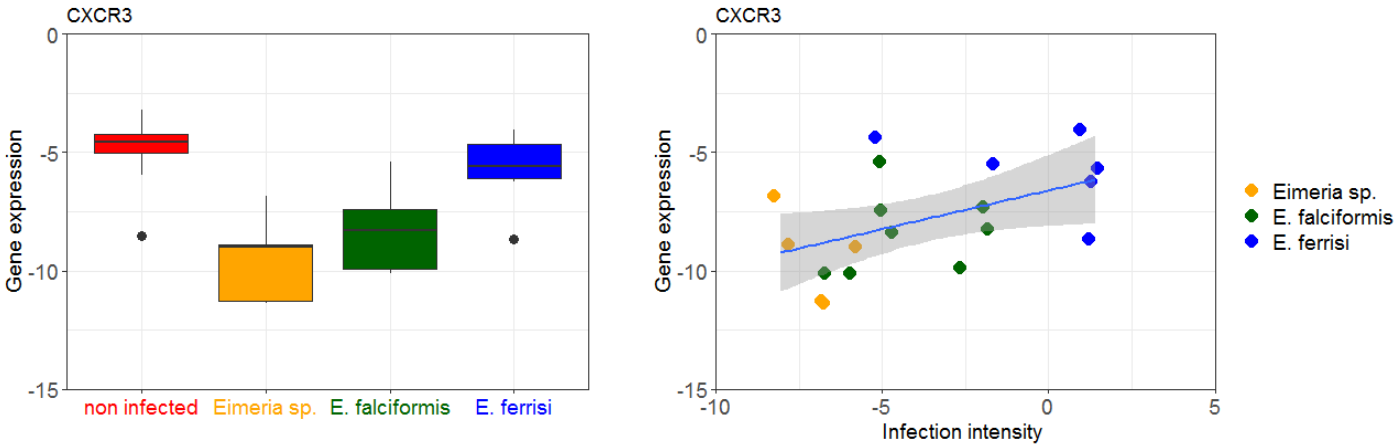
(a) **Boxplot of IL-6 gene expression** (b) **IL-6 gene expression of infected samples**

Figure 5.6: **IL-6** a) The infection types are illustrated along their infection intensity. There is a decreasing slope of the immune gene expression between the non infected and weak infected samples. The strong infected *Eimeria ferrisi* samples goes up to the immune gene expression level of non infected samples.
b) The linear model shows a significant increasing slope of the immune gene expression along the infection intensity: lm= 0,02 *



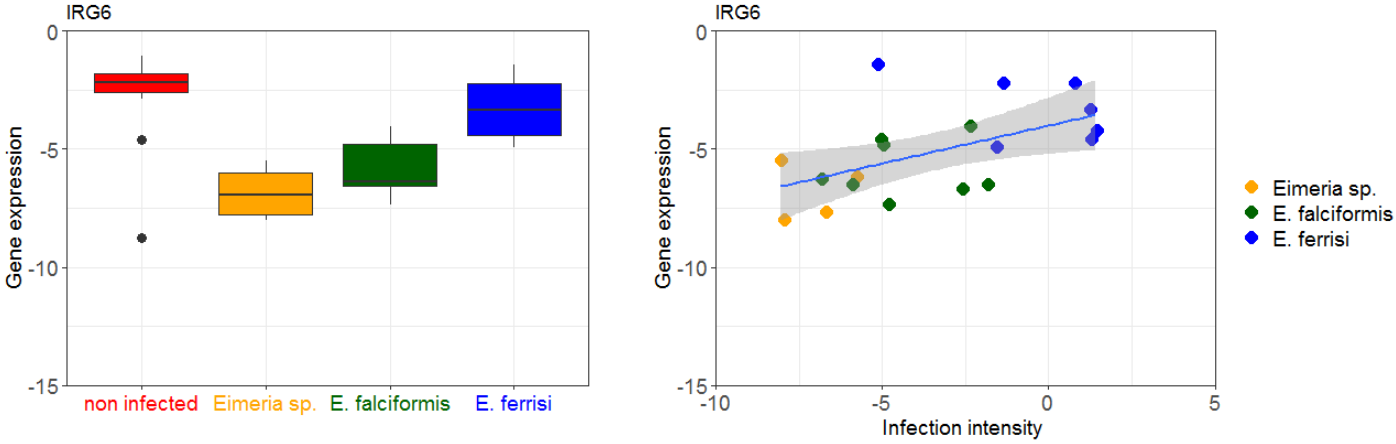
(a) **Boxplot of IL-12rb1 gene expression** (b) **IL-12rb1 gene expression of infected samples**

Figure 5.7: **IL-12rb1** a) The infection types are illustrated along their infection intensity. There is a decreasing slope of the immune gene expression between the non infected and weak infected samples. The strong infected *Eimeria ferrisi* samples goes up to the immune gene expression level of non infected samples.
b) The linear model shows a significant increasing slope of the immune gene expression along the infection intensity: lm= 0,014 *



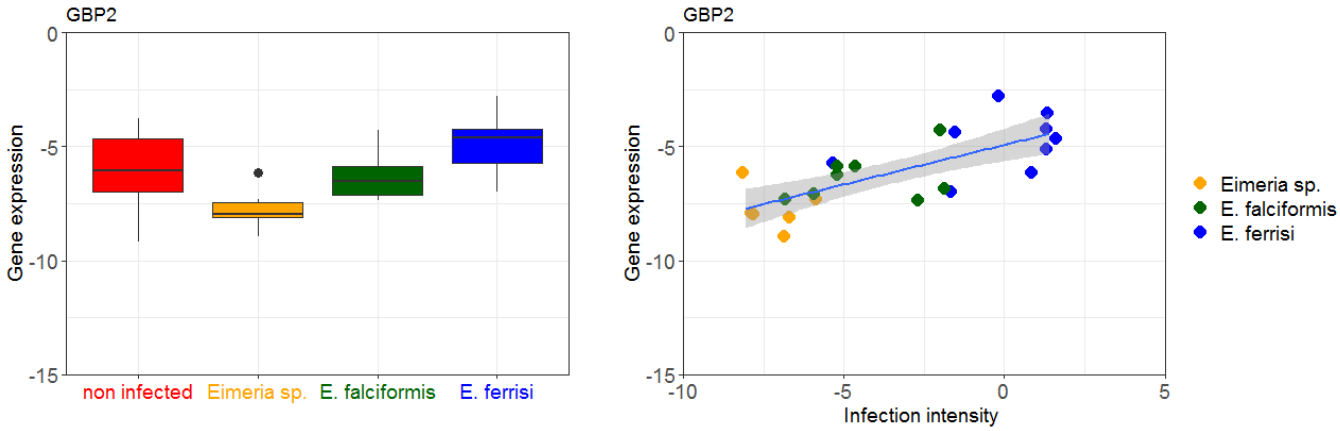
(a) **Boxplot of CXCR3 gene expression** (b) **CXCR3 gene expression of infected samples**

Figure 5.8: **CXCR3** a) The infection types are illustrated along their infection intensity. There is a decreasing slope of the immune gene expression between the non infected and weak infected samples. The strong infected *Eimeria ferrisi* samples goes up to the immune gene expression level of non infected samples.
b) The linear model shows a significant increasing of the immune gene expression along the infection intensity: lm= 0,03 *



(a) **Boxplot of IRGA 6 gene expression** (b) **IRGA 6 gene expression of infected samples**

Figure 5.9: **IRGA 6** a) The infection types are illustrated along their infection intensity. There is a decreasing slope of the immune gene expression between the non infected and weak infected samples. The strong infected *Eimeria ferrisi* samples goes up to the immune gene expression level of non infected samples.
b) The linear model shows a significant increasing slope of the immune gene expression along the infection intensity: lm= 0.018 *



(a) **Boxplot of GBP2 gene expression** (b) **GBP2 gene expression of infected samples**

Figure 5.10: **GBP2** a) The infection types are illustrated along their infection intensity. There is a decreasing slope of the immune gene expression between the non infected and weak infected samples. The strong infected *Eimeria ferrisi* samples goes up to the immune gene expression level of non infected samples.
b) The linear model shows a significant slope increasing of the immune gene expression along the infection intensity: lm= $1{,}05 * e^{-4}$ ***

The trend for all immune genes, except GBP2, shows that there is no significant variance between the immune gene expression of non infected and *Eimeria ferrisi* infected and between *Eimeria falciformis* infected and *Eimeria sp.*. The GBP2 gene expression shows no significant variance of *Eimeria falciformis* infected samples to none of the other infection types.
The immune gene expression of each target does not behave linear among the infection intensity. There is a decreasing slope between the immune gene expression of the non infected samples and the borderline *Eimeria sp.*. The linear model of the immune gene expression shows a significant increasing slope along the infection intensity within the infected groups.

5.3.3 Non metric MDS

The non metric MDS was done for all 48 samples and all targets as described in 4.5.3. Figure 5.11 shows the nMDS plot of all samples, coloured by their infection type as in figure 5.4. The stress value is 0.082, which corresponds to a good configuration of the dataset with no real risk of drawing false inferences [45]. There is a different cluster between non infected and infected samples. Specially, *Eimeria falciformis* and *Eimeria sp.* samples shows a large distance to the non infected cluster. Most of the samples, which are infected with *Eimeria ferrisi* shows the smallest distance to the non infected cluster.

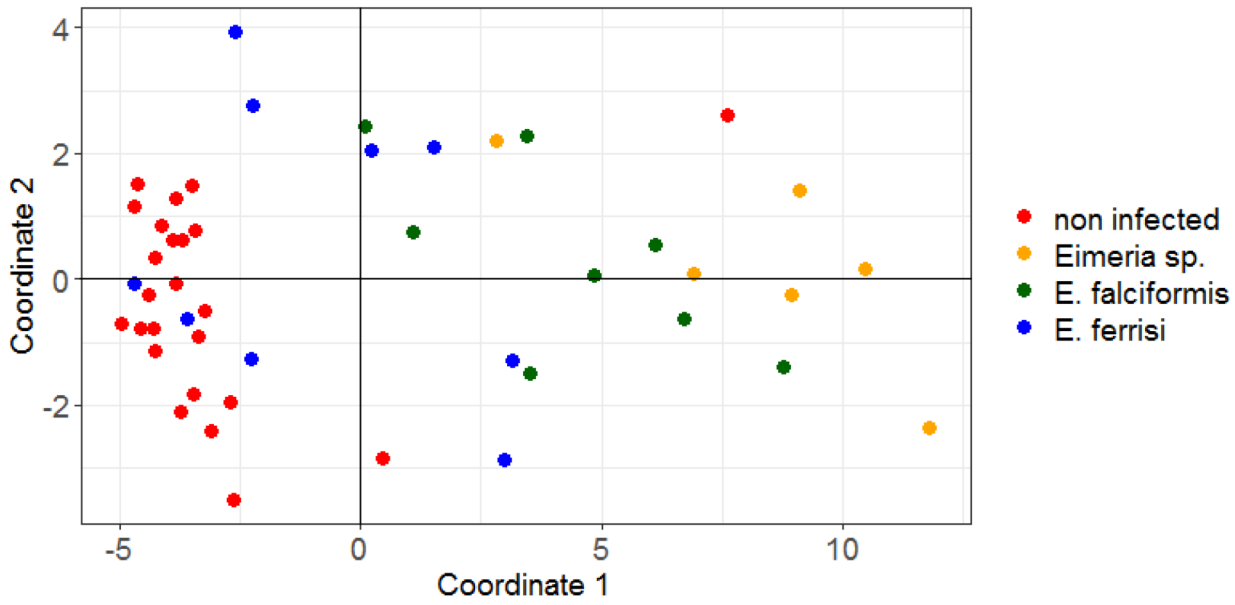


Figure 5.11: **Non metric multidimensional scaling.** The non metrtic MDS is illustrated and coloured by the infection status. The stress value is 0.082.